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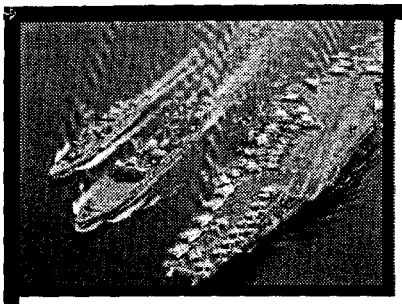
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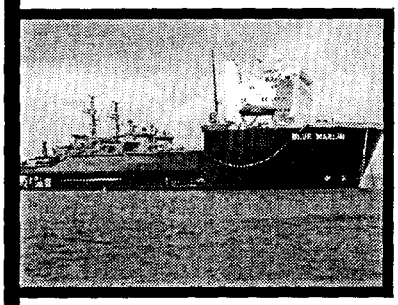
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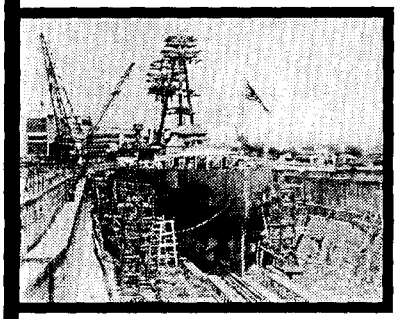
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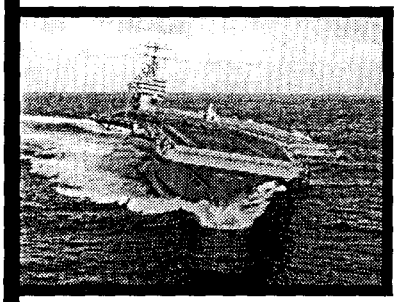
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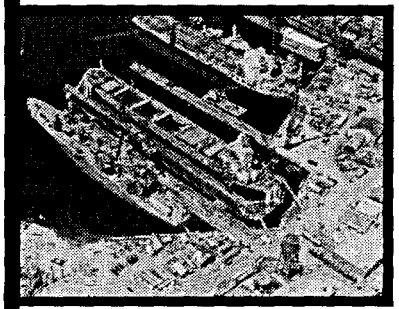
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USE OF A NAFION MEMBRANE PROBE FOR QUICK, ON-THE-SPOT DETERMINATION OF IONIC COPPER CONTAMINATION LEVELS IN NATURAL WATERS

John Foerster

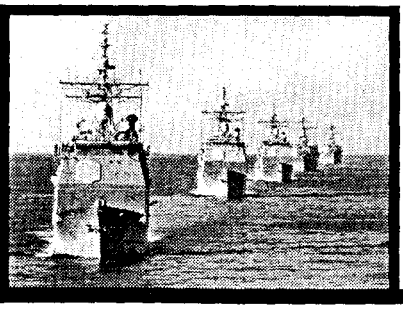
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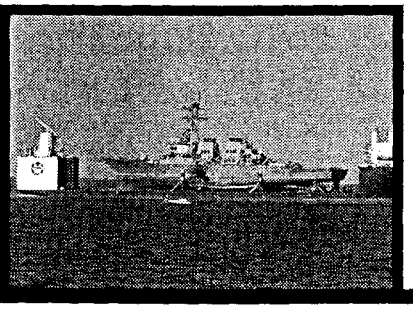
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USE OF A NAFION MEMBRANE PROBE FOR QUICK ON-THE-SPOT DETERMINATION OF IONIC COPPER CONTAMINATION LEVELS IN NATURAL WATERS

Executive Summary

A major source of trace metal contamination in the marine environment comes from the copper containing anti-fouling paints on ship hulls. This study tests the hypothesis that the organic molecule, 2,9-dimethyl-4,7-diphenyl-1,10-phenanthroline (Bathocuproine=BCP) attached to the polymer Nafion 117 is a reliable chemical for developing a sensor capable of measuring Cu(I) in seawater. The purpose is to develop a sensor system that will measure Cu (I) contamination quickly. The sensor must have

- parts per billion (ppb) detection limits,
- marine environmental immersion capability, and
- the ability to detect the copper(I) oxidation state.

An easily and readily deployed sensor can yield results that will allow the deployment of remedial methods to avert an environmental problem.

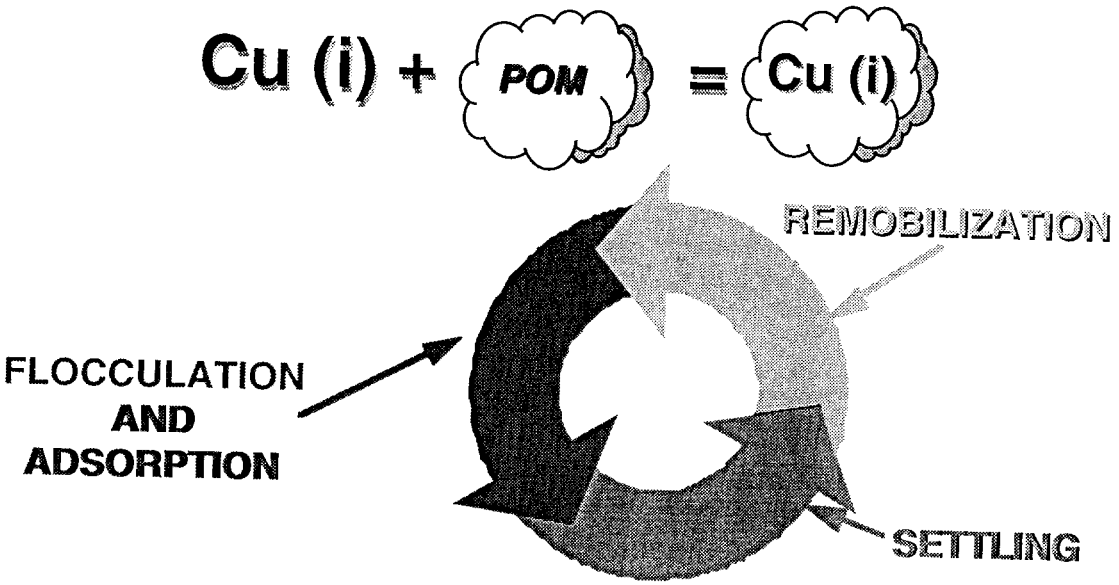
The developed sensor uses the perfluorinated ionomeric film, Nafion 117. This film has a Teflon matrix with sulfate side groups that facilitates the BCP/copper reaction. When the Nafion 117 membrane with the imbedded BCP is exposed to Cu(I), it will turn a shade of orange corresponding to the Cu(I) concentration. The developed Cu(I) concentration on the membrane is readable with a comparator, with a fiber optic sensor or with a colorimeter.

In the marine environment, anti-fouling paint containing Cu(I) presents a challenge because it is designed to leach continuously over a period of time. The Cu(I) is a biocide that kills or prevents attachment of organisms to a ship hull, but, also is a pollutional source.

The conclusions from this study are:

- that the chemical procedure using the BCP impregnated Nafion 117 membranes affords a probe to test for total available ionic copper [(I) and (II)],
- that the chemical procedures used are reproducible and robust,
- that the environment plays a role in the reaction of the probe with available ionic Cu [(I) and (II)],
- that the Nafion 117 membrane impregnated with BCP detects Cu(I), and
- that there is a knowledge base for continuing and expanding the studies.

The field use of the Nafion 117 membrane impregnated with BCP was a useful and rapid analysis tool. Further testing is needed to improve the response time.



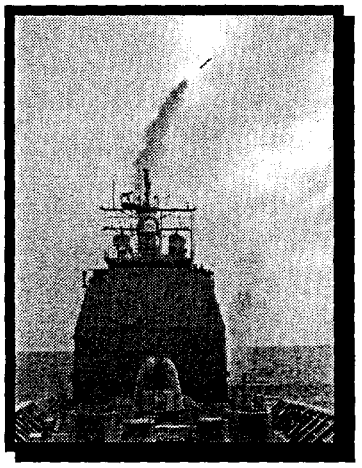
Ionic copper in the environment.

John Foerster

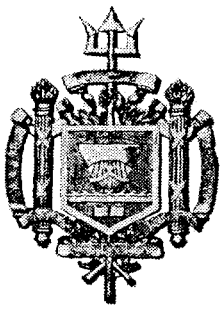
Robert Lamontagne

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INTRODUCTION









USE OF A NAFION MEMBRANE PROBE FOR QUICK ON-THE-SPOT DETERMINATION OF IONIC COPPER CONTAMINATION LEVELS IN NATURAL WATERS

by
John Foerster[1], and Robert LaMontagne[2]

I. INTRODUCTION

Being able to operate unrestricted in all navigable waters and maintain a world presence, is the highest priority of the U. S. Navy. International treaties and more stringent environmental laws impact on the Navy's ability to maintain a presence in the world and at home. With the future implementation of the Uniform National Discharge Standards (UNDS), it will be necesary to account for the ionic copper [Cu (I) and (II)] released from the anti-fouling coatings on ship hulls. Thus, it is important to have the ability to quickly and easily measure ionic copper. Compliance with these treaties and laws minimizes fines and impacts operational restrictions. The Navy's stated environmental goals are:

- to demonstrate leadership in the Federal sector by complying with Federal, state and local environmental regulations and laws;
- to prevent pollution at Navy activities;
- to cleanup shore activities where past waste disposal practices are potentially hazardous;
- to provide stewardship for natural resources on Navy activities; and
- to promote environmental protection and natural resource stewardship (NAVFAC 1998).

The use of copper releasing anti-fouling hull coatings presents a possible pollution problem.

Anti-fouling coatings prevent some and reduce other organisms (barnacles, seaweeds, tubeworms, etc.) from attaching to ship hulls. It prevents the step-wise fouling of a hull. This fouling can add to the vessel's operational cost (Figure 1). With organism attachment to a ship hull (Figure 2), comes increased drag and thus increased fuel consumption during operation (Claisse and Alzieu 1993). Wynne and Guard (1997) report that under certain conditions a ship having a 24 month biofilm can "... require 8% more shaft horsepower to move as fast as (a) comparable ship with no biofilm." Biofouling increases fuel consumption between 18 and 22% for U. S. Naval ships (Wynne and Guard 1997). The majority of navy ships use an anti-fouling coating containing copper.

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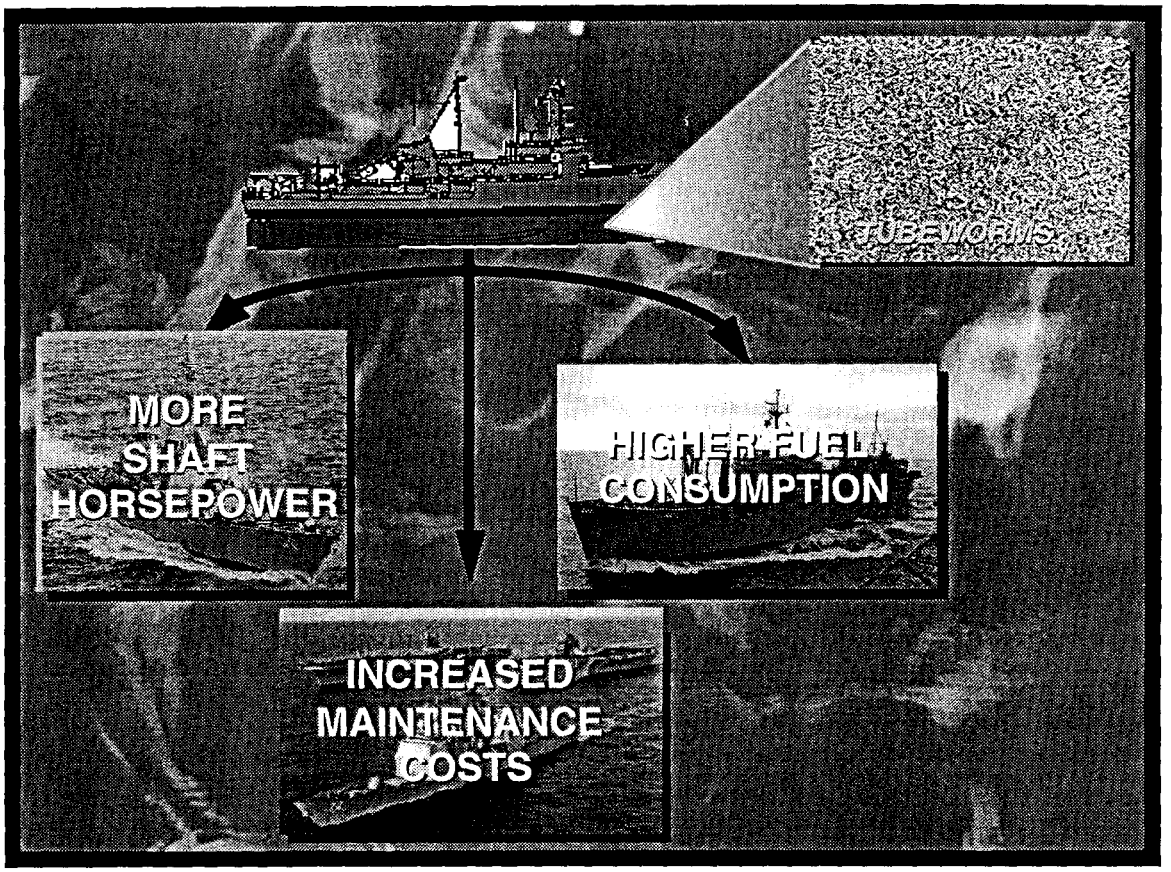


Figure 1. The cost of fouling a ship hull.

Copper can create pollution problems for non-fouling species like marine fishes (Sindemann 1996). Therefore, the purpose of this work is to develop a sensor system for use in the marine environment that can detect the initial release form of copper [Cu(I)].

The copper based anti-fouling coating is an attempt to reduce and control the fouling of a vessel. Because commercial vessels and pleasure craft are also using copper anti-fouling coatings, there is a potential for toxic loading of the aquatic environment. Nriagu (1979) reports that a major source of copper [initially Cu(I)]in the marine environment is from anti-fouling paints on ship hulls (Figure 3). Thus, this research focuses on a way to detect the levels of available ionic copper [Cu(I) and (II)] in the marine and estuarine (brackish) water environment.

Copper is a potentially toxic trace metal. At present there is no quick and easy method for determining the major ionic species of copper in the marine environment. In an effort to solve this problem, and develop a simple analysis, we test the hypothesis that the organic molecule, 2,9-dimethyl-4,7-diphenyl-1,10-phenanthroline (Bathycuproine=BCP) is a reliable chemical to use in developing a dip probe capable of measuring copper (I) ands (II) in seawater. Our purpose is the development of a sensor system that will measure trace metal contamination quickly. This will allow the deployment of remedial methods to avert an environmental problem.

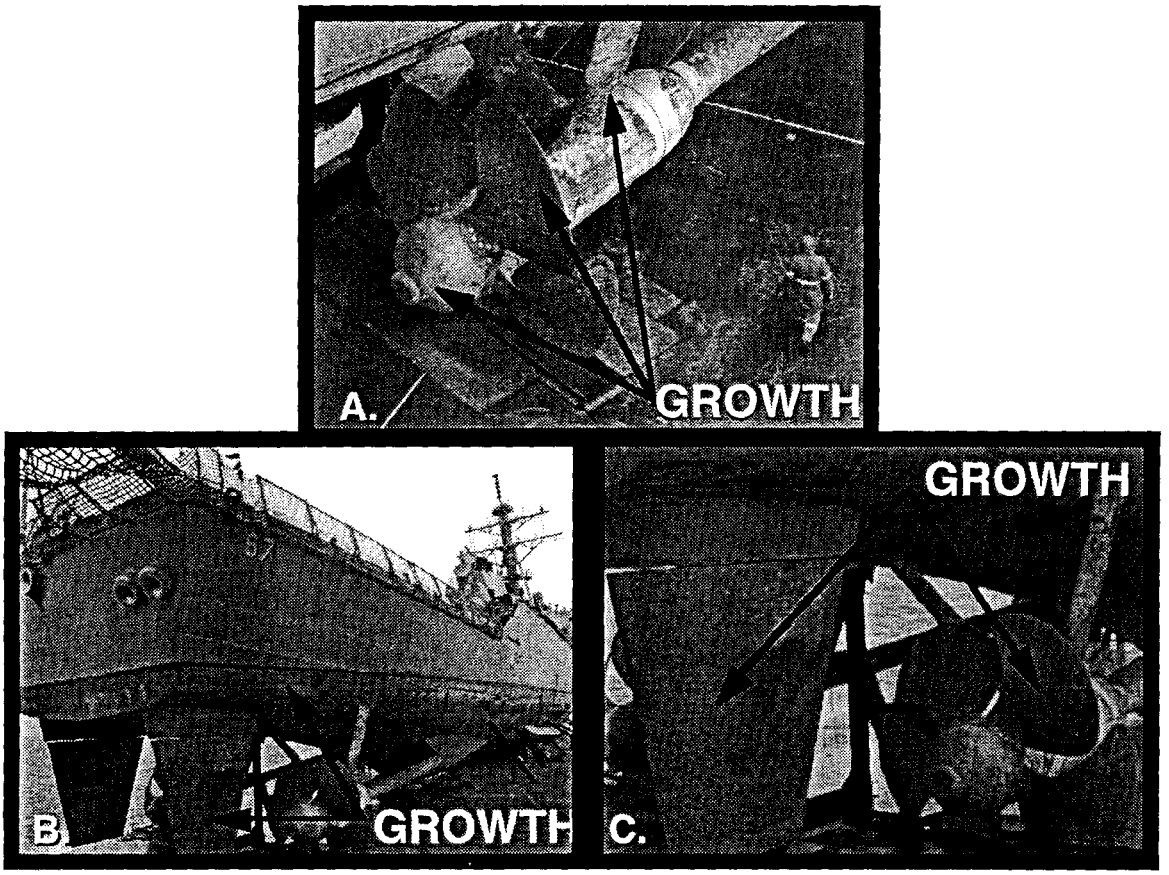


Figure 2. Shipboard bio-fouling occuring even in areas of strong currents.

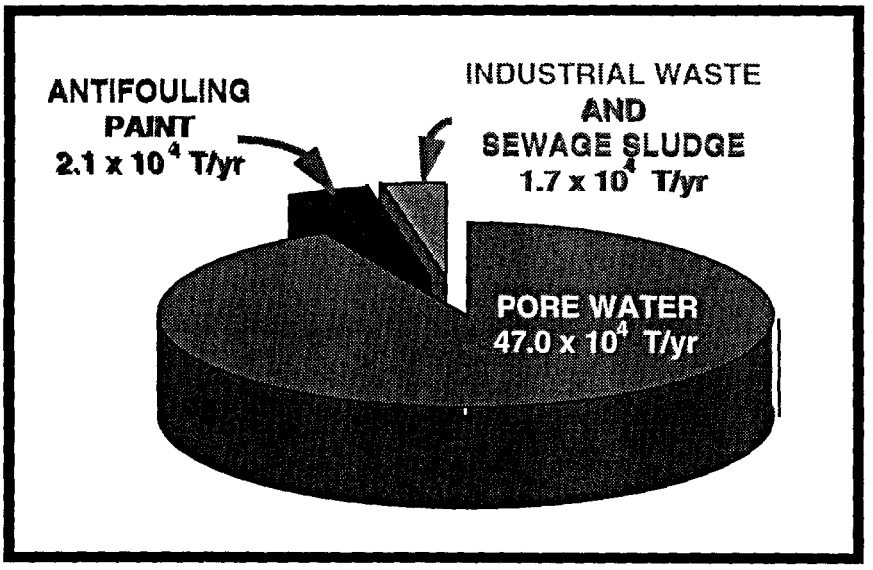


Figure 3. Major environmental sources for copper in the marine environment.

Any trace metal sensor system for use in the marine environment must have

- ppb detection limits,
- in situ operation capability, and
- the ability to detect the copper (I) oxidation state.

The Water Quality Control Act of 1965 (Paragraph 3, Section 10) reads:

"Standards of quality established pursuant to this subsection shall be such as to protect the public health or welfare, enhance the quality of water and serve the purposes of this Act. In establishing such standards the Secretary, the Hearing Board, or the appropriate state authority shall take into consideration their use and value for public water supplies, propagation of fish and wildlife, recreational purposes, and agricultural, industrial, and other legitimate uses."

Generally, water quality standards must enforce the goal that no portion of a water body (bay, harbor, estuary, etc.) solely receives and transports wastes. This then leads us to defining our "receiving" body of water as one that is capable of sustaining life while being useful for Navy purposes. In general, water is:

- a necessity of life,
- a transporter of disease,
- a coolant,
- a cleanser,
- a diluent,
- a navigational highway,
- a recreational pastime and aesthetic resource,
- a harvestable food resource,
- a source of energy,
- a refuge,
- a nursery for wildlife,
- a home for biological pests and nuisances,
- a sink and source for civilization's wastes, and
- a hazard for life and property (rewritten from Mackenthun 1969).

Thus, water has different meanings for different users, and its quality affects the direct use and the aquatic life that inhabits it.

1. Copper in the Environment

Many researchers have noted the potential toxic effects of trace metals (Sorensen 1995, Vymazal 1995, and Newman and McIntosh 1991). Copper is the primary "pesticide" ingredient in anti-fouling hull paints on Navy ships. The copper leaches from the hull coating, and is a potentially toxic trace metal waste. As a toxic substance, it will eliminate aquatic biota until

- dilution,
- dissipation,
- chemical change, or

• volatilization

reduces the toxic concentrations below some population's and individual's response threshold. This results in development of tolerant species and/or contaminated food resources.

Figure 4 illustrates the effect of a non-toxic waste like sewage on bottom organisms as the waste transports away from the source. Total numbers of animals decrease in the zone of impact. After dissipation and dilution of the waste, the numbers of individuals recover.

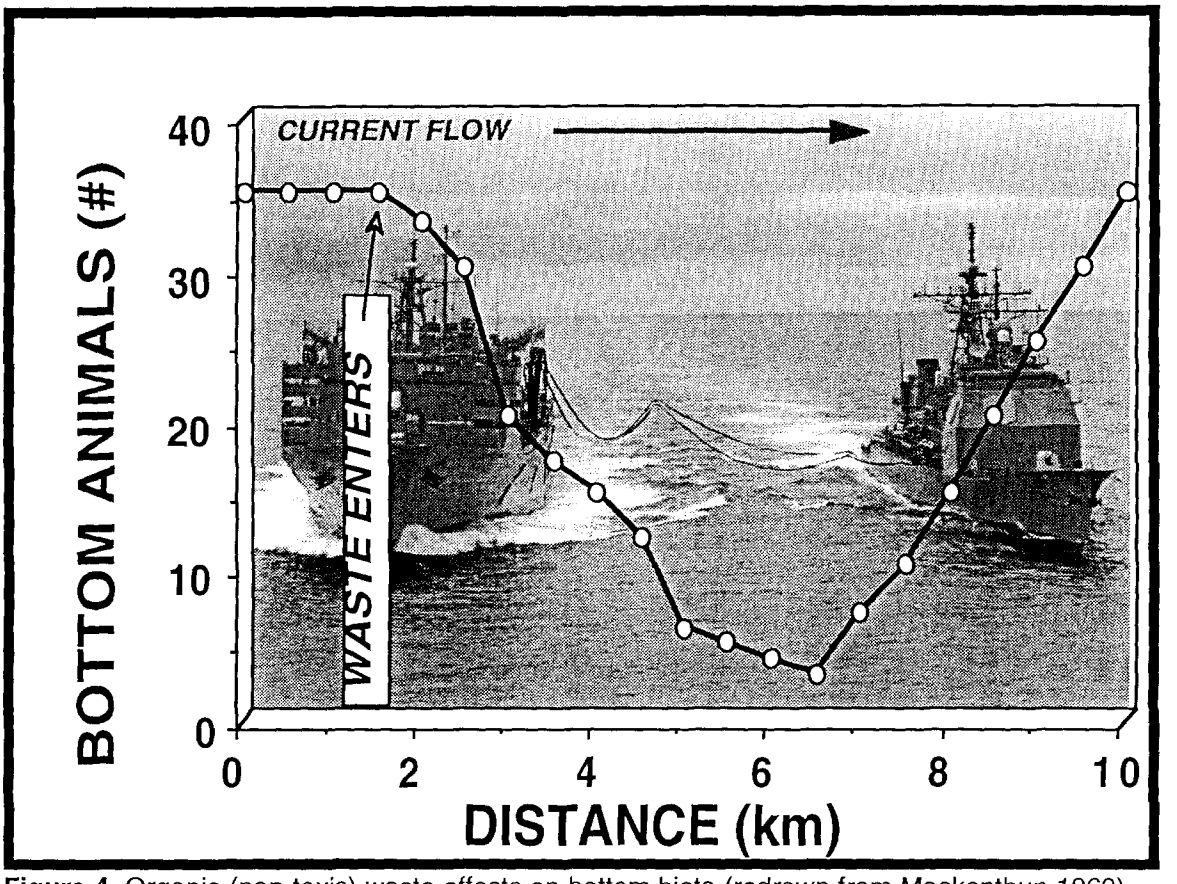


Figure 4. Organic (non-toxic) waste effects on bottom biota (redrawn from Mackenthun 1969).

On the hull of a ship, a surface area is readily available for colonizing. Resultant colonization is biofouling and it is what the anti-fouling coating is designed to retard. Biofouling is a stepwise procedure (Figure 5). It begins with the unfouled surface being conditioned chemically and the formation of an organic film (Little 1984, Loeb and Neihoff 1975, Baier 1972). This film "wets" the unfouled surface making it more attractive for colonization by bacteria (Dexter et al 1975, Dexter 1978). Once the surface is organically "wet" then bacteria can attach (reversible sorption) but are removed easily (Marshall 1976, Marshall et al 1971). As soon as the bacteria colonize, they develop extracellular bridging polymers. These accumulate (irreversible sorption) and the primary biofouling film forms (Little 1984). This primary film becomes attractive to algae and protozoa as well as attracting detritus and corrosion

products (Little 1984). With the algae and protozoa in the fouling film, the next steps are the attraction of animals like barnacles and tube worms (Figure 5).

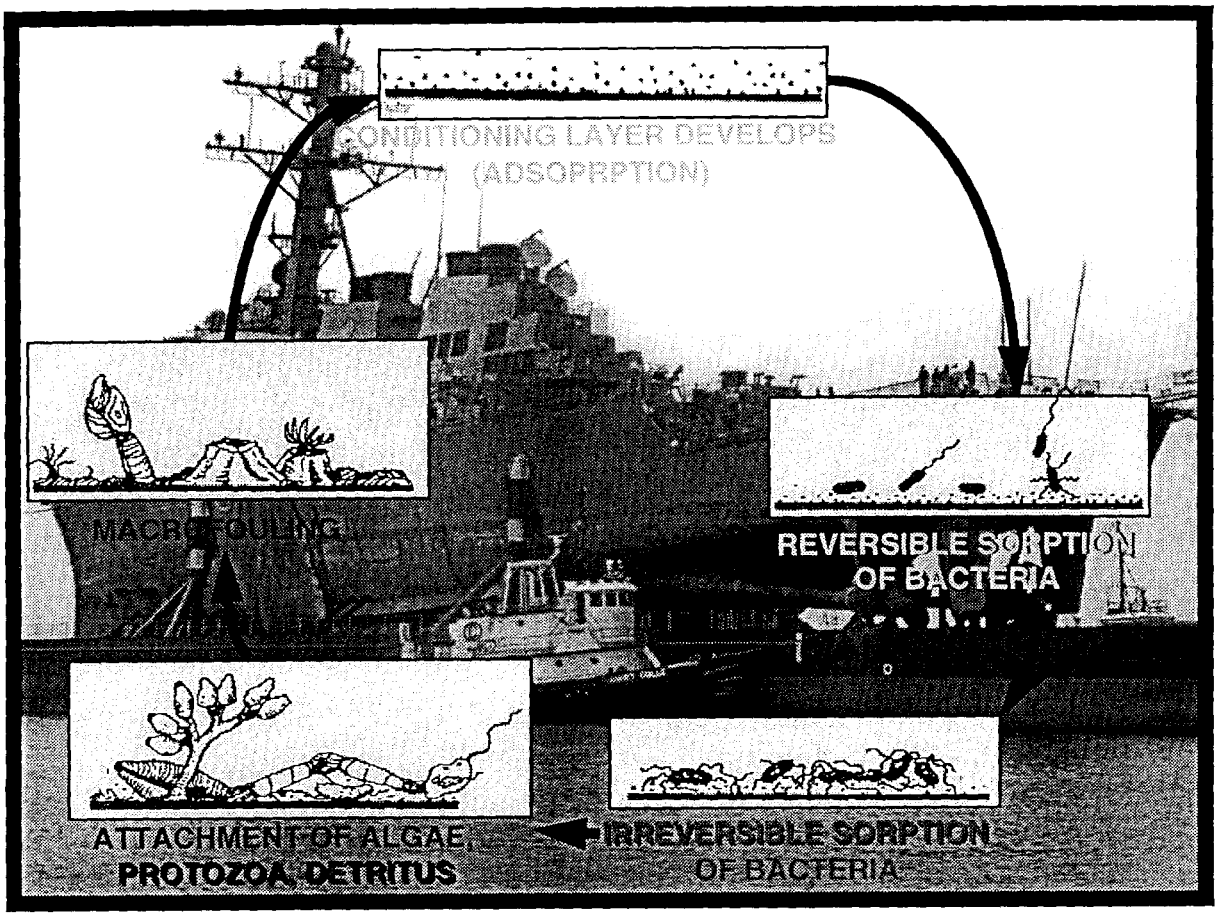


Figure 5. Step-wise bio-fouling of ship hull.

Any unfouled surface placed in the marine environment invites colonization. This colonization occurs slowly or rapidly depending on the ambient temperature. In essence, the entire biofouling process is an ecological succession process (Odum 1971). The initial biofilm and bacteria stages are pioneer succession and the later progression through algal and protozoan inhabitation are sere stages (stepwise changes) until the biofouling film reaches a climax. Climax is where if you scrape off a barnacle what will grow back is a barnacle. Anti-fouling coatings are an attempt to stop or at least slow down this process on any structure or vessel placed in marine waters. The effect of the ant-fouling coating ameliorates with distance from the source as long as the input volume remains relatively constant.

2. Copper Chemistry

Copper has an atomic number of 29. It is the first element of subgroup IB of the periodic table, and has low chemical reactivity (towards the Nobel end of the electrochemical series), while being electronegative to hydrogen (Wakeman

1954). The electronic structure is $1s^2 2^2 2p^6 3s^2 3p^6 3d^{10} 4s^1$, and the $4s^1$ electron has a low ionization potential. This means the electron removes easily to give the cuprous ion [Cu^+ = Cu (I)]. The ionization potential of the $3d^{10}$ shell is only slightly higher resulting in the formation of the cupric ion [Cu^{++} = Cu (II)]. Standard oxidation reduction potentials (Barnard 1954) are:

$$Cu^0 <----------> Cu^+ + e^-, -0.52 volts$$
$$Cu^+ <----------> Cu^{++} + e^-, -0.15 volts$$
$$Cu^{++} <----------> Cu^{+++} + e^-, -0.18 volts$$

These potentials are important in assessing the reaction of the copper in an aqueous environment. The aqueous biogeochemistry of copper depends on

- the chemical composition and concentrations in the receiving water (calcium, magnesium, other metal species, oxygen, potassium, sodium, organics),
- the physical (pH, redox, salinity, temperature) and hydrodynamic characteristics (currents, mixing) of the receiving water,
- the chemical form of the copper in the receiving water (i.e., complexed, dissolved, solid phase, etc.), and
- the biological dynamics of the organisms in the receiving water [uptake (age, disease, size, physiology, life style), consumption, adsorption, absorption, etc.].

In general, the biogeochemistry of copper in the marine environment follows the paths outlined in Figure 6.

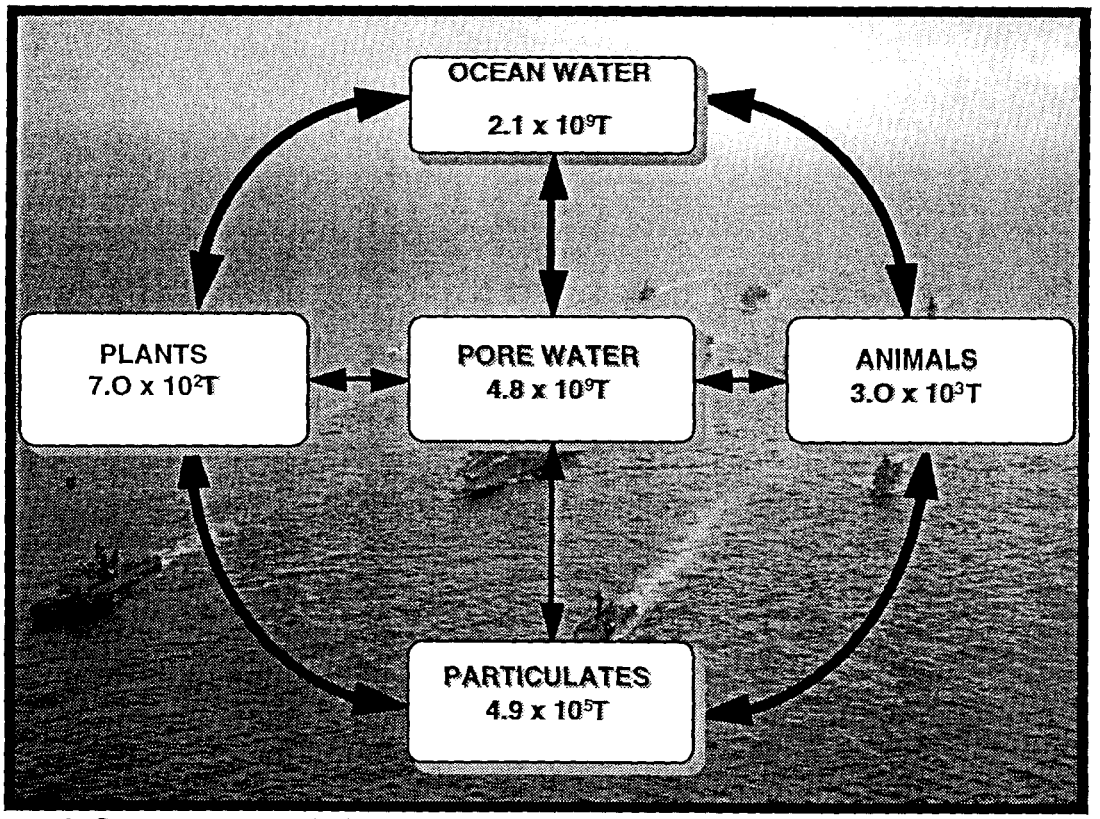


Figure 6. Ocean copper cycle (data from Nriagu 1979).

3. General Information

Figure 7 is the Global Copper cycle as reported by Nriagu 1979. The pore water of the sediments tends to be the sink for copper while waste and anti-fouling paints are anthropogenic sources. Because of its reactivity, ionic copper quickly gets complexed. As such, any analysis for ionic copper must be made quickly or the ionic copper becomes complexed (Hall et al 1989).

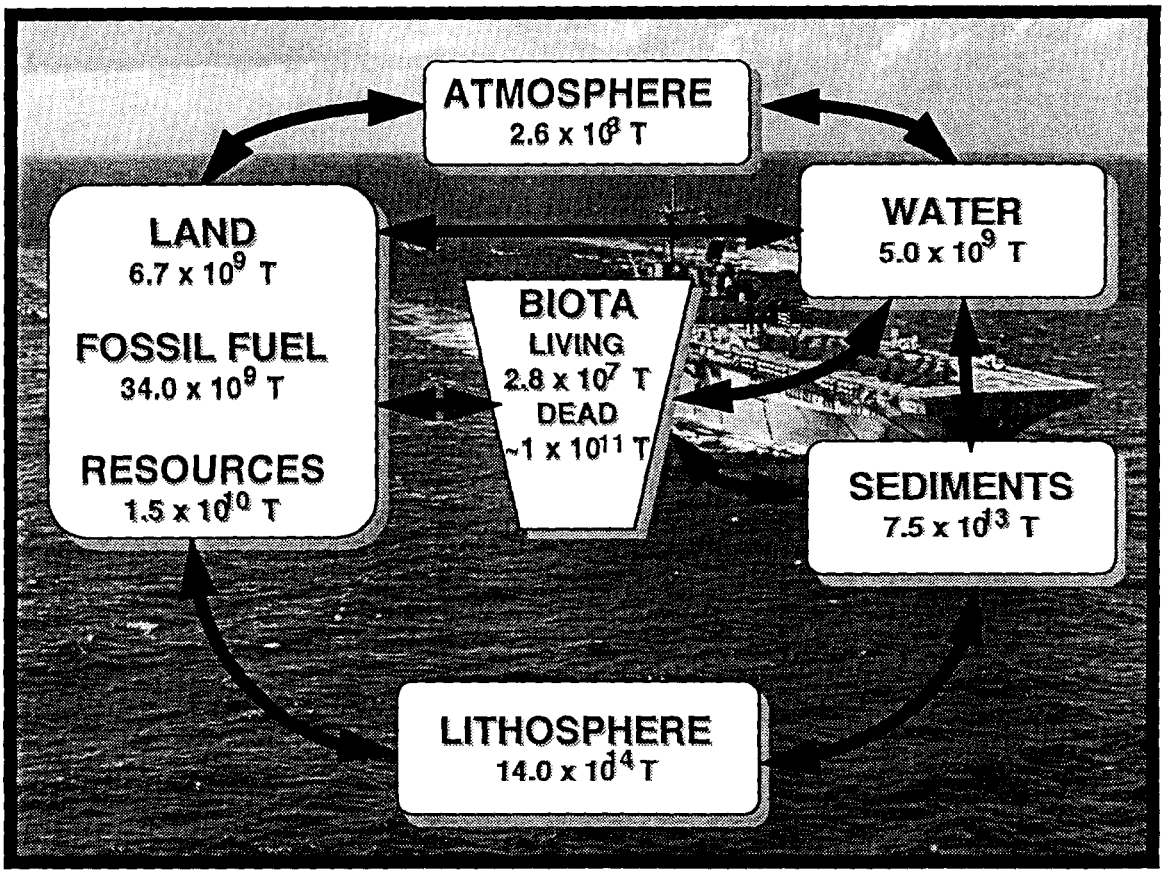


Figure 7. Global copper cycle (redrawn from Nriagu 1979).

4. Toxicology

Copper is an essential and required nutrient in the picogram to low microgram range (Newman and McIntosh 1991, Sorensen 1991). Plants require copper for

- plastocyanin (a copper containing protein important for electron transport in photosynthesis,
- enzyme co-factor, and
- catalyzing the direct oxidation of various organic compounds.

Copper in animals is important in:

- thirty enzymes and glycoproteins (i.e. amine oxidases, catalase, cytochrome oxidase, dismutase, uricase, etc.) (Williams 1969, Aaseth and Norseth 1986, Goyer 1986),
- hydrogen peroxide/organic substance destruction and energy production (Sorensen 1991),
- promotes iron absorption,
- involved in iron transport from tissue to plasma,
- assists in maintaining the myelin of the nervous system,
- important in the formation of brain and bone tissue, and is
- necessary for hemoglobin synthesis.

Levels of copper in the body receive constant trace inputs from external sources and from internal storage. When levels of copper from external sources exceed species specific levels, defense mechanisms, and toxic effects result. Because of the importance in metabolic functions, copper toxicology is more complicated than nonessential or xenobiotic (purely toxic) trace metals like mercury. In the face of stabilized environmental conditions, toxicity of copper depends on whether the copper is a free Cu(I) or Cu (II) ion, or complexed in some form such as copper carbonate, copper chloride or copper hydroxide. Most copper research report efforts relative to copper (II) (Ervin 1995).

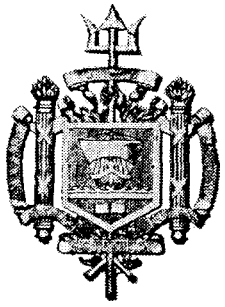
The toxicology of copper depends on the organism and thus data may be highly variable in the same species in the same environment. Data (Foerster et al 1994, Sorensen 1995, Vyczmal 1991) demonstrates that copper toxicity is relative to:

- life history stage (egg, larva/fetus, pre-adolescent, adolescent, adult),
- environmental factors,
- ages,
- sex,
- size,
- starvation,
- trace metal tolerance (prior exposure),
- activity,
- protection mechanisms (i.e., metallothionein)

These effects depend on whether the copper is reacting antagonistically, synergistically, or additively. In copper anti-foulant paints, zinc is an additional trace metal whose action is additive to the copper in producing the overall anti-foulant effect.

MATERIAL AND METHODS

MATERIAL AND METHODS









II. ANALYTICAL METHODS

To be an effective anti-foulant in the marine environment, copper needs to be in its ionic form (Cu (I) or Cu (II)). Copper in the ionic form is very poisonous for aquatic plant photosynthesis (Steemann Nielsen and Wium-Andersen 1970, Sunda and Gillespie 1979), and aquatic animal life (Newman and McIntosh 1991, Sorensen 1995). The overall protection in the marine environment against these toxic ionic forms is the presence of organic compounds. Organic compounds (humic acids, siderophores, etc.) form complexes with copper and thus remove the copper toxicity (Steemann Nielsen and Wium-Andersen 1970, Alexander and Corcoran 1967, Moffett et al 1997). Keeping the ionic forms available in the face of salinity, pH shifts and temperature changes is the challenge that anti-foulant coatings must overcome to be economically successful and biologically effective. In addition, there is the challenge of copper rapidly hydrolyzing to form copper hydroxides and copper carbonates (Zirino and Yamamoto 1972).

There are a variety of methods available for the study of copper in the aquatic environment. These include stripping voltammetry (Clavell and Zirino 1985, Xue and Sunda 1997), atomic absorption (Zuehlke and Kester 1985, Clesceri et al 1998), inductively coupled plasma (Clesceri et al. 1998), optical fibers (Ervin et al 1993), piezoelectric sensors (Nomura et al 1997), ion-selective electrode (Vuceta and Morgan 1977), and specific dye chemistry (Bjorklund and Morrison 1997, Clesceri et al 1998, Moffett et al 1985, Diehl and Smith 1972, Blair and Diehl 1961, Borchardt and Butler 1957, Smith and Wilkins 1953). For this study it was important to identify a simple (both in method and time), robust and reproducible procedure. To do this, an ionic copper detecting probe was developed using an ionomeric membrane (Nafion 117) inbeded with a copper (I) detecting dye (BCP).

1. Chemical Procedures

This study used the reaction of copper (I) with an organic dye molecule Bathocuproine (BCP), 2,9-dimethyl-4,7-diphenyl-1,10-phenanthroline (Figure 8), to produce a color in proportion to the amount of copper (I) complexed.

According to Leckie and Davis (1979), the unique property of Cu(I) was its ability to form organometallic bonds. Reaction with the BCS/BCP dye (Smith and Wilkins 1953) was the beginning of establishing a simple analytical procedure for determining the presence and quantity of ionic copper. Both BCS and BCP react with Cu(I) to produce an orange color. This color was measurable with a spectrophotometer at 484nm (Figure 9a), or a colorimeter (Figure 9b) using a green filter in the range 470-500nm.

The Bathocuprione Method (Greenberg et al. 1998) was the control test method used to compare the response of the dip probe. A single Cu(I) molecule reacts with 2 BCS or BCP molecules (Diehl and Smith 1972). The BCS/BCP molecules, due to stearic hindrance, are at right angles to each other

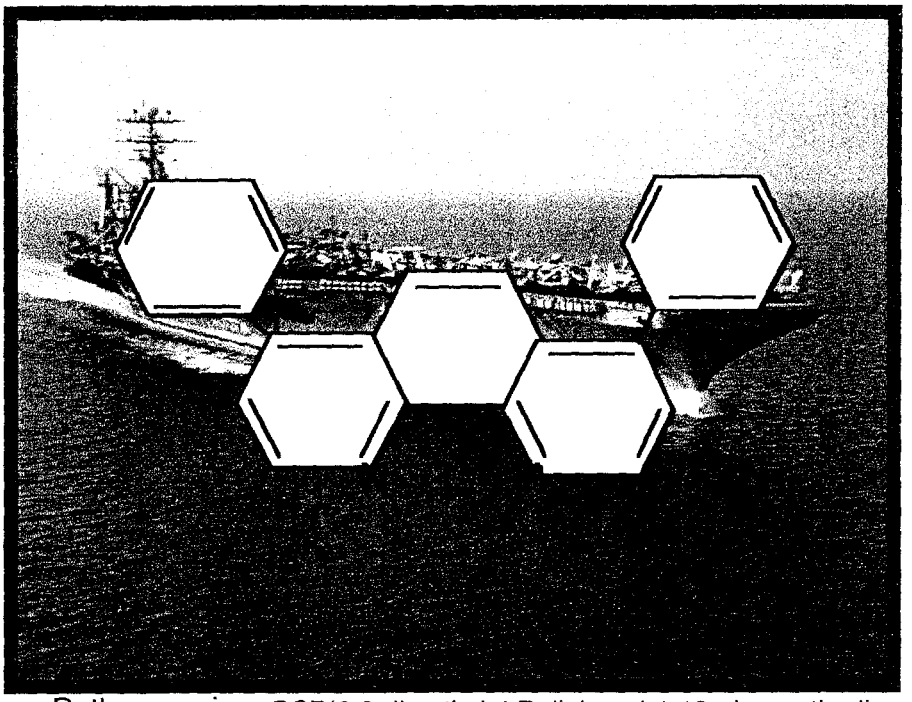


Figure 8. Bathocuproine=BCP(2,9-dimethyl-4,7-diphenyl-1,10-phenanthroline (redrawn from Diehl and Smith1972).

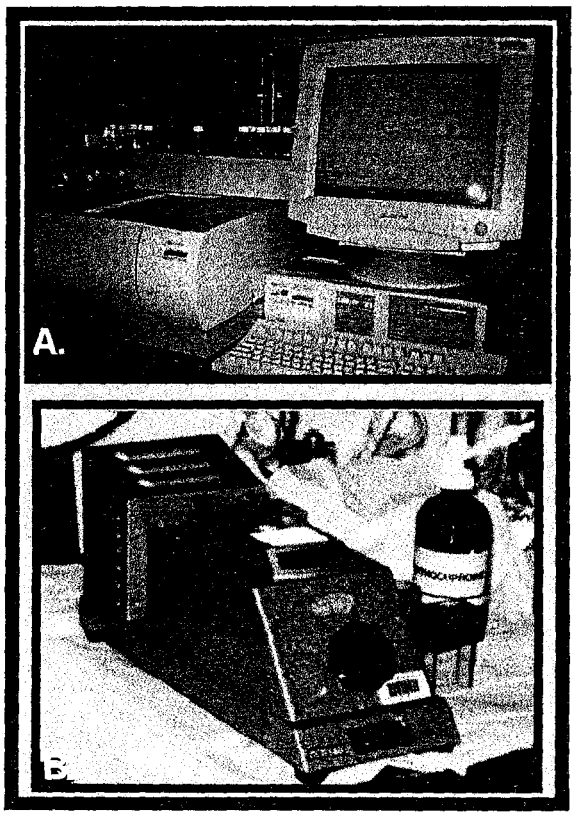


Figure 9. Set-up for measuring the results of the copper (I) reaction. A=spectrophotometer; B=colorimeter.

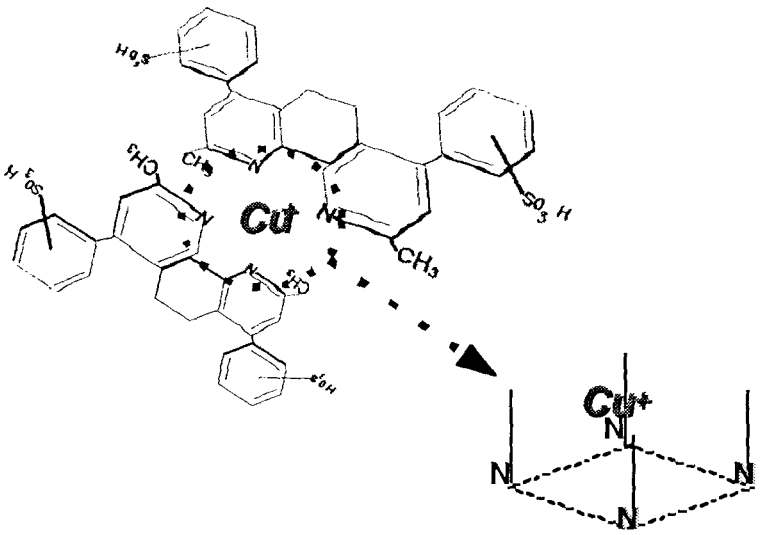


Figure 10. Copper (I) complexed between the nitrogens of BCS molecules that are at right angles to each other.

To establish the quantity of Cu(I) and Cu(II) in the water samples, a differential procedure was used. To measure for Cu(I) only BCS/BCP was added to a buffered sample. In a second sample, the complete BCS/BCP method with the hydroxylamine hydrochloride reductant was added and the total ionic copper concentration measured. The difference between the values obtained with the complete method and with just BCS/BCP is Cu(II).

2. Calibration

The calibration procedure involved preparing standard curves for each type of water (seawater, distilled water) (Figure 11). Known amounts of copper (I) were added in the following parts per billion (ppb=ug/L) concentrations, 5 ppb; 10 ppb; 25 ppb; 50 ppb; 100 ppb; 1000 ppb. All testing used standard copper concentrations prepared with a 1000 ug/ml standard in 2% nitric acid from SPEX Industries, Inc.

Comparison of the curves in Figures 11 demonstrates a method that:

- reads in the low ppb range,
- reads in the high ppb range,
- gives similar results in chemically different water,
- appears chemically robust, and
- produces a reasonable agreement with Beer's Law (Hemond and Fechner 1994) over concentration range of 10 - 2000 ppb.

Therefore, the goal of this study was to develop a dip probe for detecting copper (I) in seawater. Copper (I) in solution was developed by

- reducing a Cu(II) standard to Cu (I),
- leaching copper (I) from Interviron anti-fouling red paint, and from Woolsey Neptune Red.

The copper was leached from coatings on glass coupons (Figure 12).

The coupons are a source of copper (I) for experiments that included:

1. leach rate studies
    1a. in seawater
    1b. in distilled water
    1c. in pond water
2. copper (I) to copper (II) conversion, and
3. probe studies

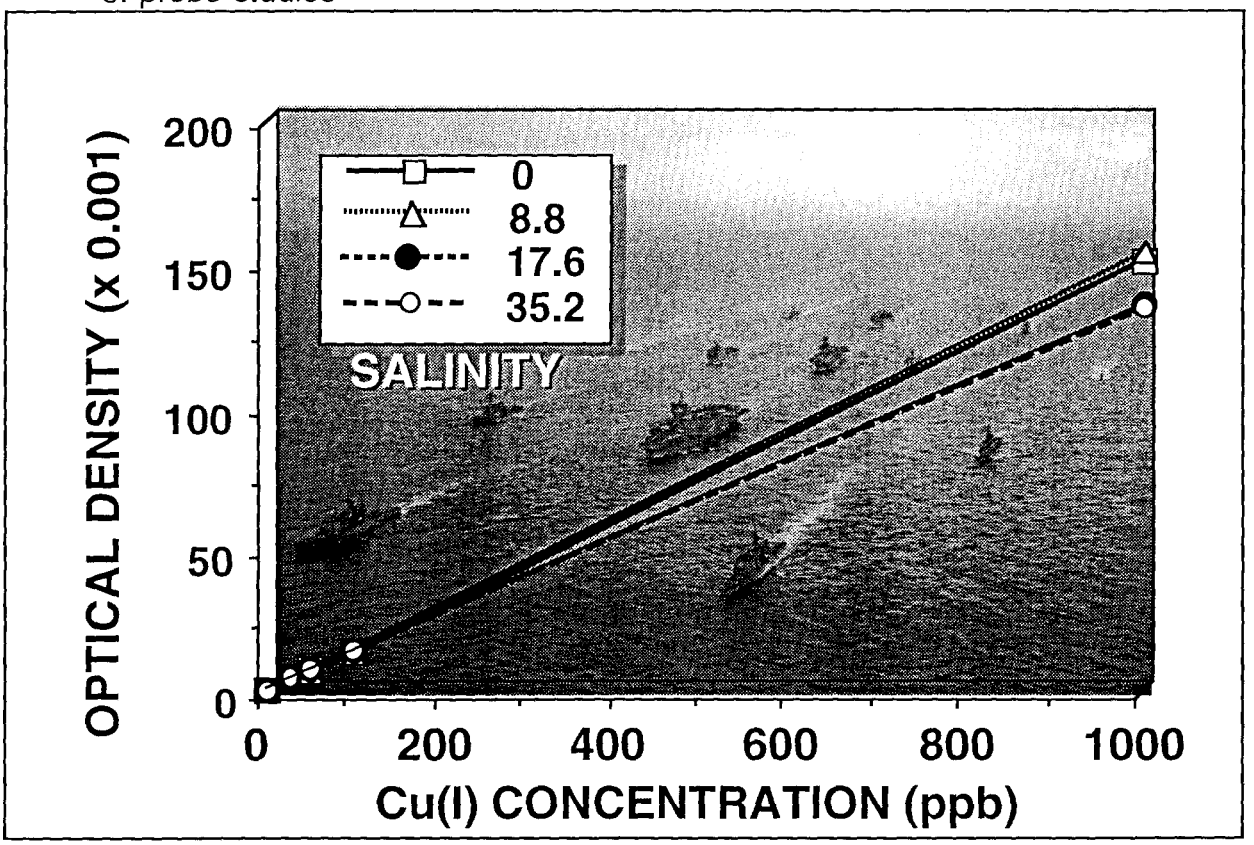


Figure 11. Calibration curves for various salinity waters.

3. Probe Development

To develop the dip probe, a perfluorinated ionomeric film matrix (Nafion 117) was impregnated with the BCP reagent. Present studies indicate that the ionomer interactions with electrolytes like ionic copper depend not only on..."the solvent but also on the type of ionic groups in the polymer, the type of counterions, and the pH (Kruczala and Schlick 1999)." Structurally, the film is a Teflon backbone having sulfate side groups (Figure 13). As an ionomer, it has both hydrophilic and hydrophobic sites. It is expected that the BCP molecules reside within the hydrophobic site.

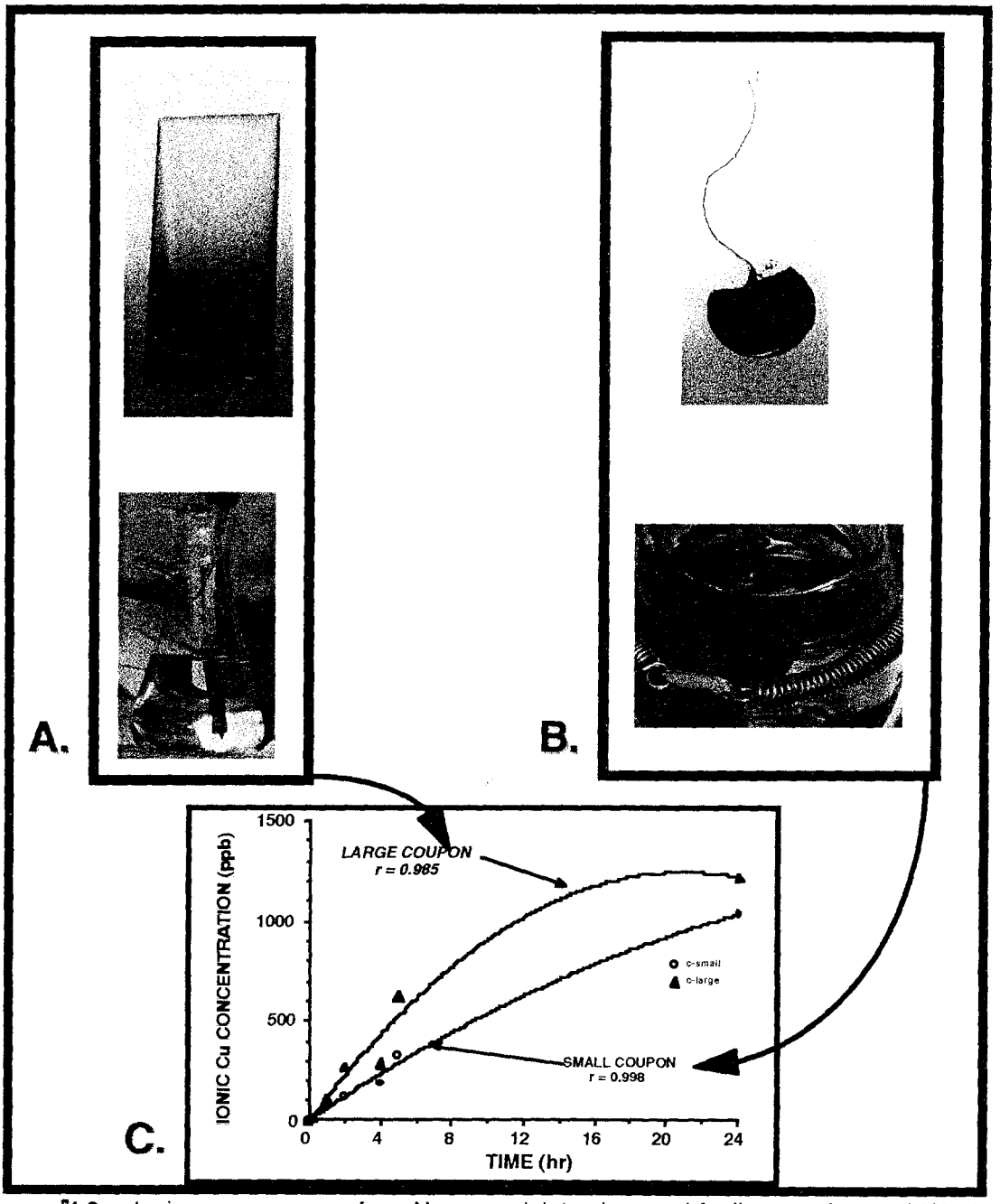


Figure 12. Ionic copper source from Navy used Interviron anti-fouling coating and Woolsey Neptune Red. A=Glass coupon with Interviron; B=Glass coupon with Woolsey Neptune Red; C=Leaching response of glass coupons with copper containing anti-fouling coating.

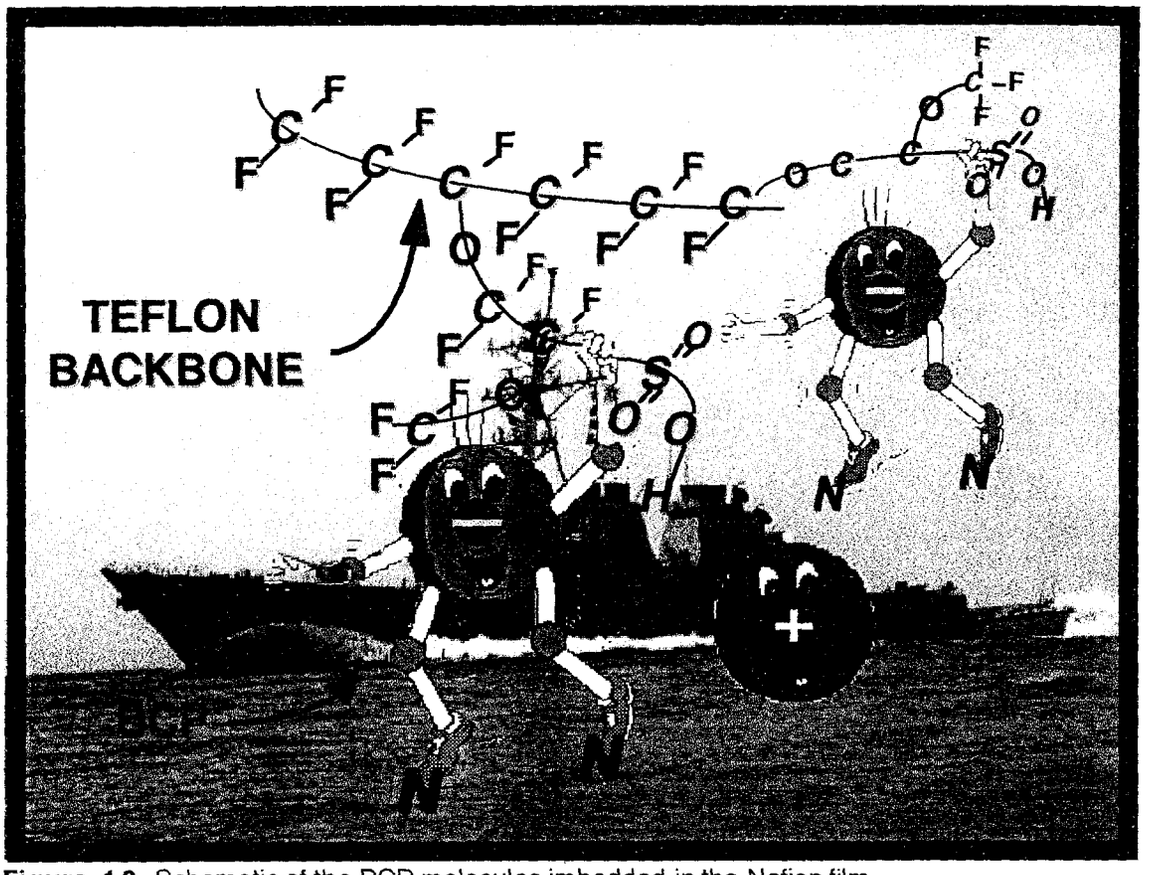


Figure 13. Schematic of the BCP molecules imbedded in the Nafion film.

Initially, Nafion was cut into 2x3cm pieces. The procedure for imbedding the BCP was as follows:

(1) Cut Nafion film into usable strips (2 x 3 cm).
(2) Place Nafion film strips into 1 molar nitricacid.
(3) Boil the Nafion strip in nitricacid for 0.5 hr.
(4) Remove strip and rinse with deionized water.
(5) Soak strip for 12 hours (overnight) in deionized water.
(6) Prepare 10^{-3} (0.001) molar solution of bathocuproine (BCP) in 100% ethanol.
(7) Obtain small screw cap vials and fill with BCP/ethanol.
(8) Place one strip of nitricacid boiled Nafion into each vial with the BCP/ethanol solution.
(9) Leave strip in vial for 24 hr.
(10) After 24 hr. remove strip and rinse in deionized water.
(11) Place strip in 1000 ppb Copper(I) solution (500 ppb copper standard in 50ml deionized water + 1ml 50% HCl + 5ml hydroxylamine hydrochloride + 5ml sodium acetate [see *Standard Methods*]).
(12) Leave strip in Cu solution for 6-24 hours. The membrane will turn orange.
(13) Remove the strip, rinse in deionized water, remove color in 1 molar nitricacid (1hr); remove and rinse strip in deionized water.

(14) Store preconditioned BCP/Nafion strips in deionized water until use.
(15) To regenerate strip after use, place in 1 molar nitric acid until color fades, rinse in deionized water and store in fresh deionized water until re-use.

The operational theory is that their exists:

- a source of copper (I),
- a method to read the concentration of copper (I), and
- a device to tabulate the amount.

Once activated with BCP, the membranes received testing in both the laboratory and the field. Calibration of the membranes followed the same procedure as that for water samples. The calibration device for the membranes was the Klett-Summerson Colorimeter using the 4.4cm light path cell (Figure 14) using a specifically designed membrane holder (Figure 15). Primarily, testing was on the reaction of the membrane.

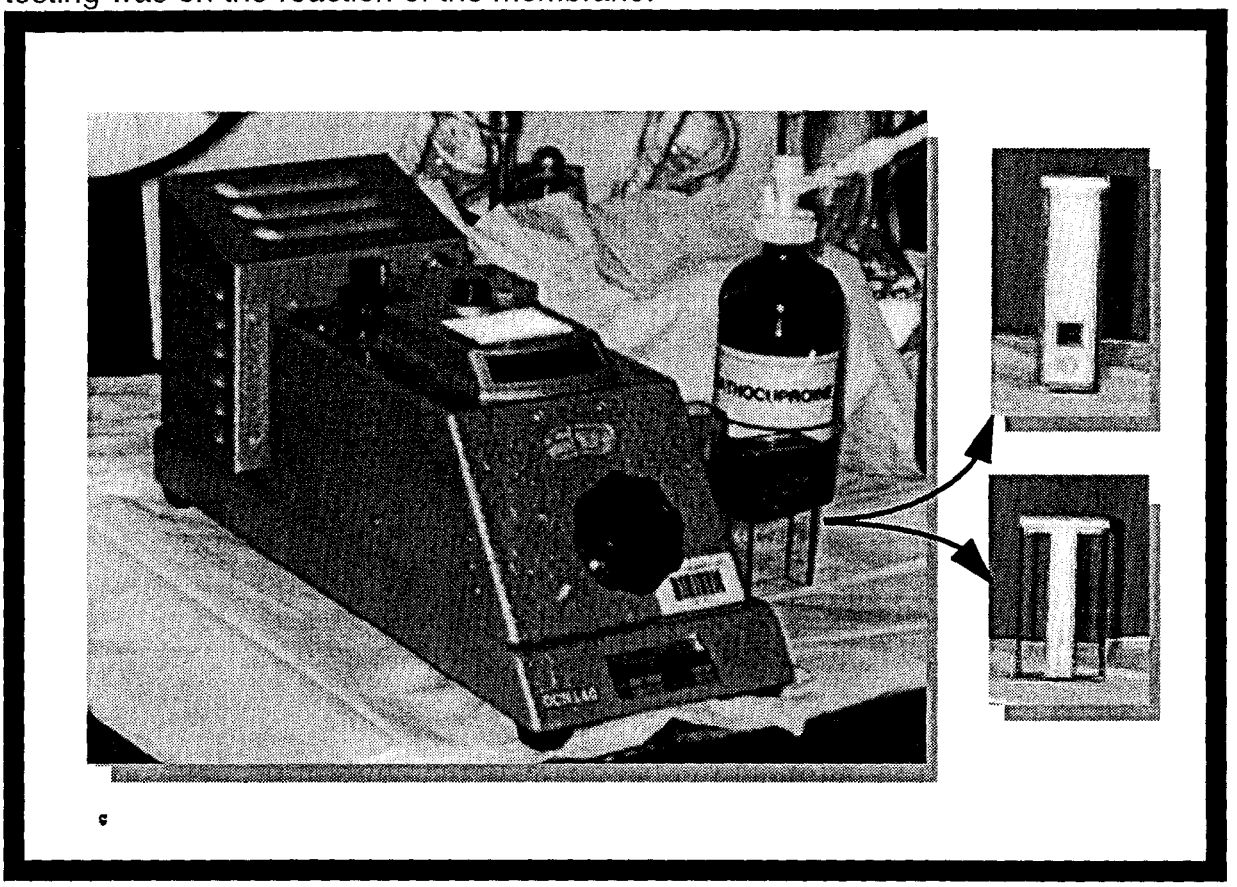


Figure 14. Colorimeter with 4.4cm cell and teflon membrane holder.

Membrane probes were prepared by the investigators and commercially by the LaMotte Chemical Company of Chestertown, Maryland. The LaMotte Chemical Company was contracted to see if the methods developed in the laboratory could be

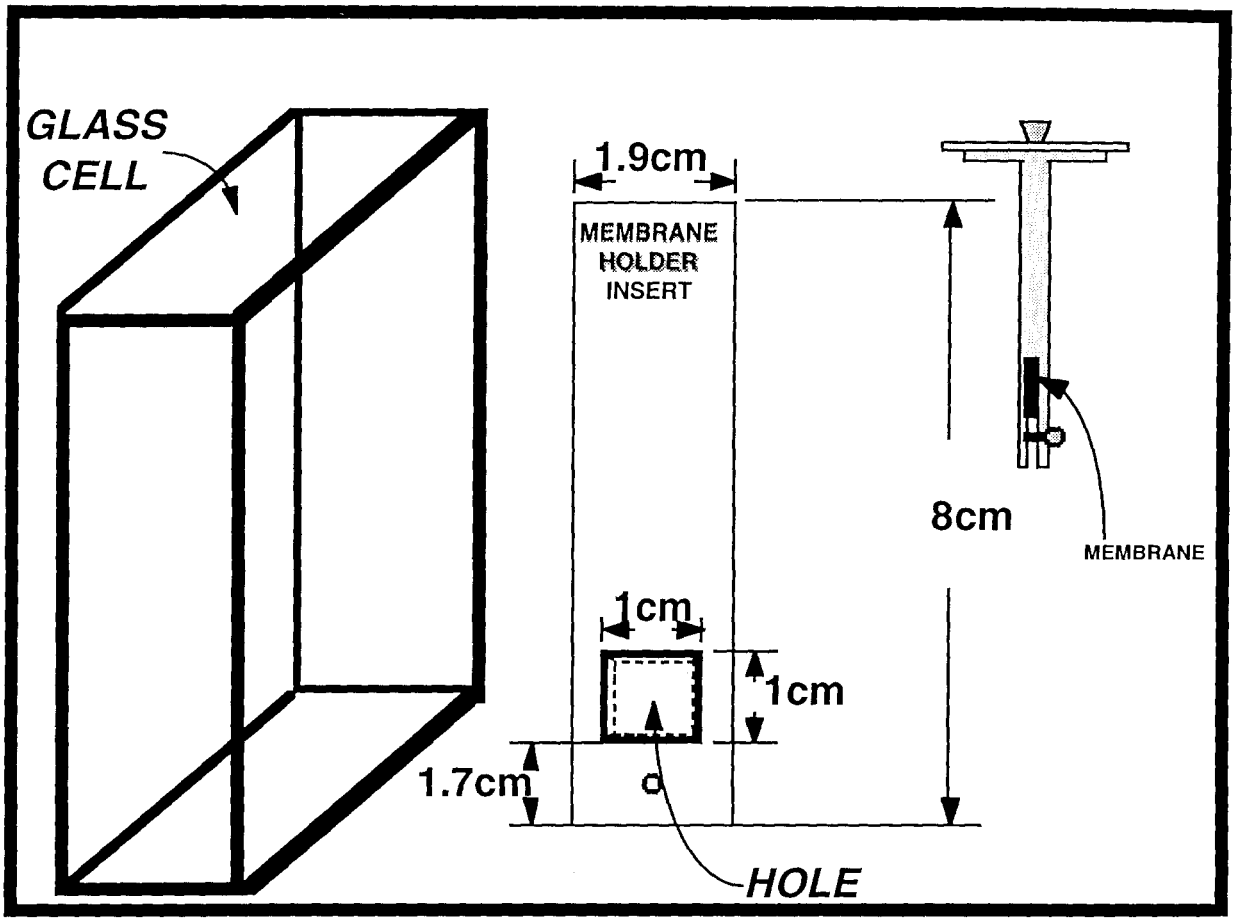


Figure 15. Specially constructed Teflon membrane holder used to test Cu(I) uptake into Nafion membrane.

successfully commercialized. Membranes were prepared by the LaMotte Chemical Comapny with the following variations:

- razor cut
  - pre-testing not activated with Cu(I) (see preparation method)
  - post-testing activated with Cu(I) (see preparation method)
- punch cut
  - pre-testing not activated with Cu(I) (see preparation method)
  - post-testing activated with Cu(I) (see preparation method)
- stored in de-ionized water only
  - pre-testing not activated with Cu(I) (see preparation method)
  - post-testing activated with Cu(I) (see preparation method)
- stored in fungal retardant preservative
  - pre-testing not activated with Cu(I) (see preparation method)
    - -- ethyl alcohol
    - -- chloroform
    - -- Kathon CG[5-chloro-2methyl-4-isothiazolin-3-one and 2-mthyl-4-isothiazolin-3-one])
  - post-testing activated with Cu(I) (see preparation method)
    - -- ethyl alcohol
    - -- chloroform

-- Kathon CG[5-chloro-2methyl-4-isothiazolin-3-one and 2-mthyl-4-isothiazolin-3-one])

The general testing scheme for the membrane probes is in Figure 16.

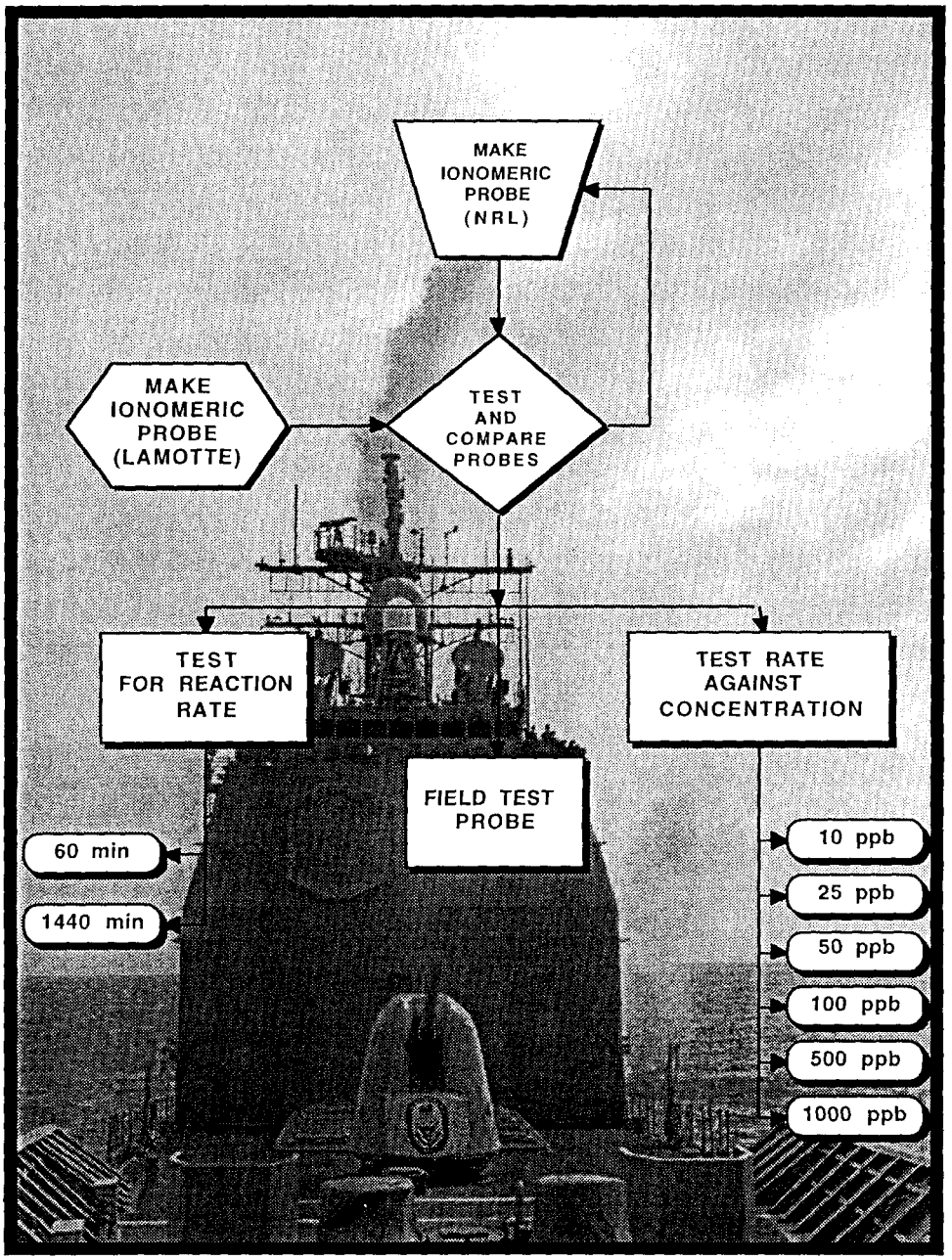


Figure 16. General test scheme for ionomeric polymer ionic copper testing probes.

4. Testing Water

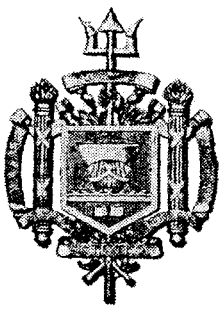
The seawater used in all chemical procedures, leach rate studies, and for testing the membrane was from the Sargasso Sea (center of North Atlantic Ocean gyre). This water was free of detectable copper and filtered to remove particulate organic matter. All the seawater passed through 0.45 micron Millipore cellulose filters to remove organisms and particulates. The filtering

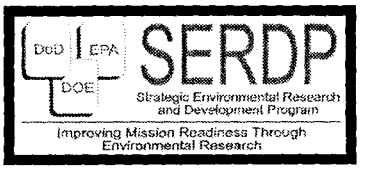
set-up used a water trap to prevent any back flushing of tap water during filtration. Storage of the filtered water was in nitric acid cleaned bottles. Distilled water was from tap water passed through a Millipore, Inc. Milli-Q and Milli-RO deionization system and analyzed for the presence of copper. In addition, some tests used locally obtained pond water. The pond water was for use in the leach rate experiments. This water, after filtering with 0.45 micron Millipore filters, received storage in nitric acid cleaned containers. In addition, artificial seawater was used. It was prepared from Instantocean salts.

Cleaning of all glassware was with a 10% solution of nitric acid. Distilled water rinsed glassware went into a nitric acid cleaning solution overnight, rinsed in the Millipore deionized water and soaked for 24 hours in Millipore deionized water before drying and storage. There was an on going constant check of all experimental water and glassware for contamination by copper.

RESULTS AND DISCUSSION

RESULTS AND DISCUSSION









III. RESULTS AND DISCUSSION

Because of the reactivity constant, ionic copper reacts quickly with ligands in the aquatic environment (Leckie and Davis 1979). The processes affecting the concentration and distribution of ionic copper are:

1. Physical
    - 1-a. Diffusion
    - 1-b. Advection
    - 1-c. Sedimentation

2. Chemical
    - 2-a. Volatilization
    - 2-b. Neutralization
    - 2-c. Precipitation
    - 2-d. Flocculation
    - 2-d. Adsorption
    - 2-e. Desorption
    - 2-f. Dissolution
    - 2-g. Oxidation
    - 2-h. Reduction
    - 2-I. Geochemical

3. Biological
    - 3-a. Response
    - 3-b. Toxicity
    - 3-c. Stimulation
    - 3-d. Incorporation
    - 3-e. Accumulation
    - 3-f. Degradation

Ionic copper released into the aquatic environment follows the pathways listed in Figure 17. These pathways illustrate the reactivity of ionic copper as well as its ability to move into all areas of the marine environment. More specifically, Figure 18 demonstrates how Cu(I) reacts as it circulates through the aquatic habitat.

Therefore, the goal of this study was the development of a robust, accurate and precise method to measure ionic copper in the marine/estuarine environment. To achieve this goal, a probe was developed using Nafion 117 ionomeric membranes impregnated with BCP. Membranes were impregnated with BCP, and subjected to copper (I) concentrations. Membranes were produced in the laboratory at the Naval research Lab and by the LaMotte Chemical Company. All membrane preparation followed the method outlined in the Materials and Methods (Section II). As a calibration check, the results of the

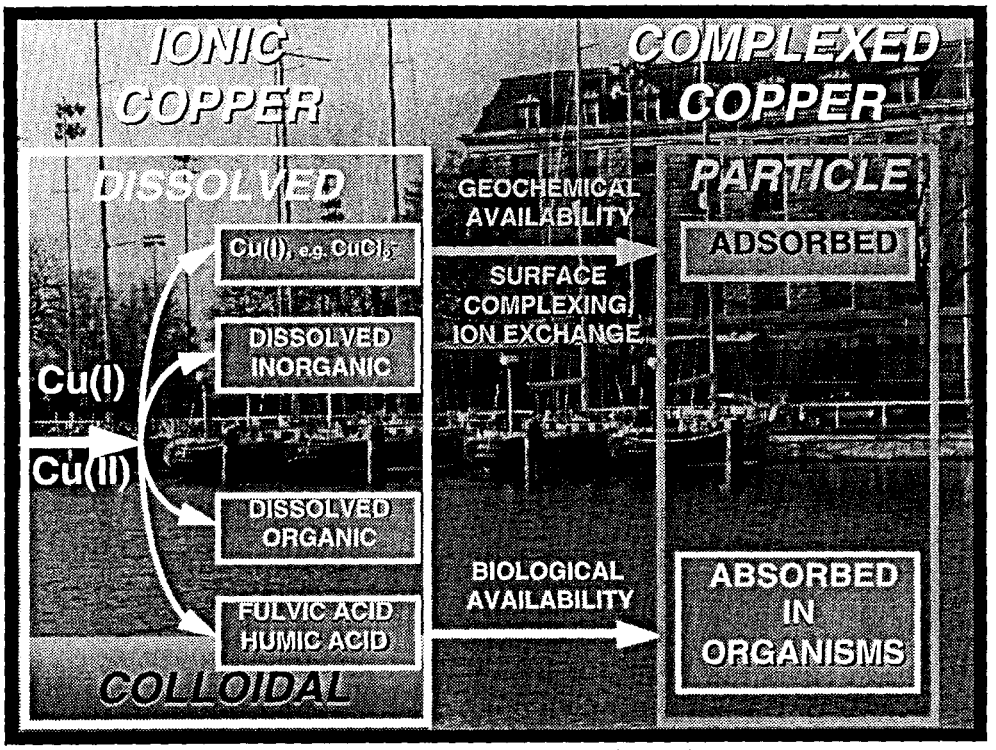


Figure 17. Reaction pathways for ionic copper in the aquatic environment.

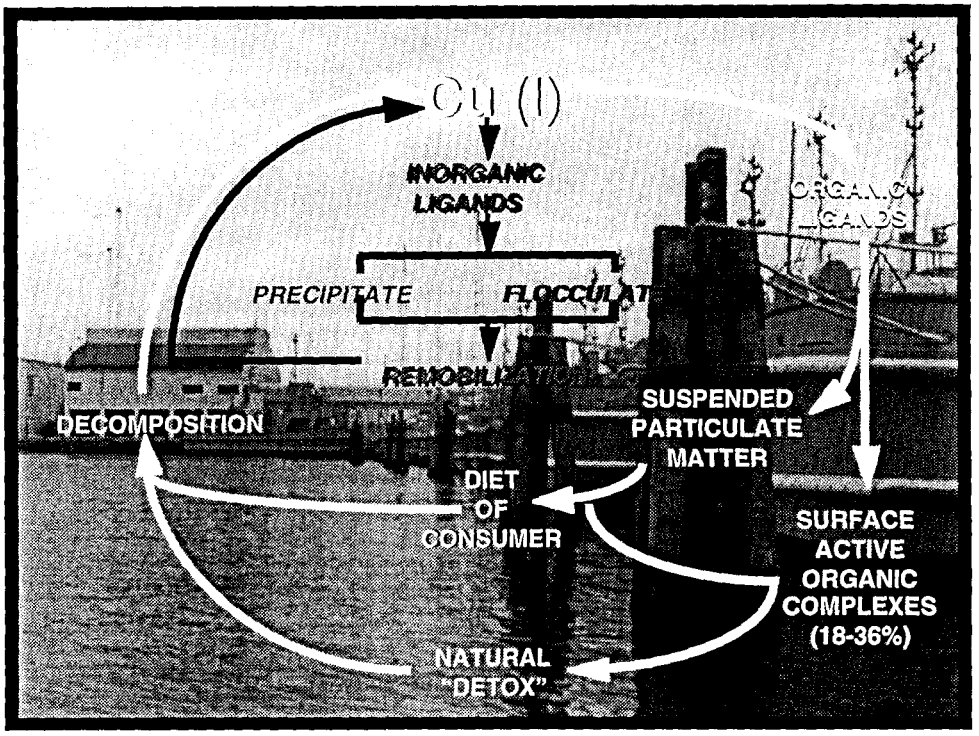


Figure 18. Cu(I) circulation through the aquatic habitat.

membrane study were compared to the Bathocuproine Standard Method (Clesceri et al 1998). Results of the comparison between the reference analysis

and the membrane analysis (Figure 19) showed that the membrane method reads within 17% of the Standard Method.

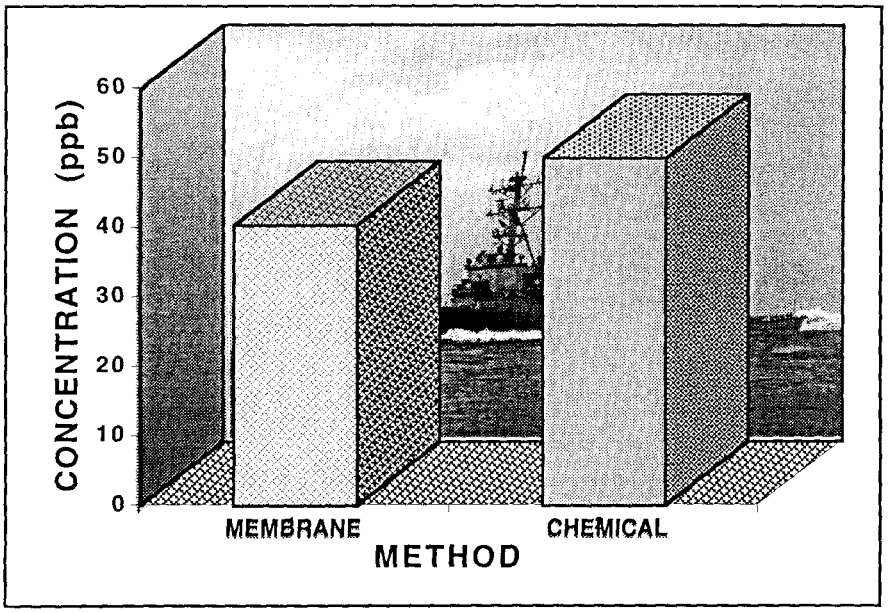


Figure 19. Comparison of the result from testing the Nafion 117 membrane impregnated with BCP to the Bathocuproine Method (Clesceri et al 1998).

1. Standardization Tests (Standard Method)

To develop a reproducible, accurate and precise method to measure copper in the marine environment, the method must have robustness. This means the end products resist change over time and consistent testing yields standard curves that do not deviate. The reference method was the "Standard Method" developed by Smith and Wilkins (1953) and codified by nationwide testing in the handbook *Standard Methods for the Examination of Water and Waste Water* (Clesceri et al. 1998). Figure 11 were the curves derived from multiple testing of water samples with the "standard" method in various salinities.

Comparison of the curves in Figure 20 demonstrates a method that:

- reads in the low ppb range,
- reads in the high ppb range,
- gives similar results in chemically different water,
- appears chemically robust, and
- produces a reasonable agreement with Beer's Law (Hemond and Fechner 1994) over concentration range of 10 - 1000ppb.

Salinity appears to affect the uptake of Cu(I) by the ionomeric membrane (Figure 20). There tends to be an initial uptake, then a retardation of Cu(I).

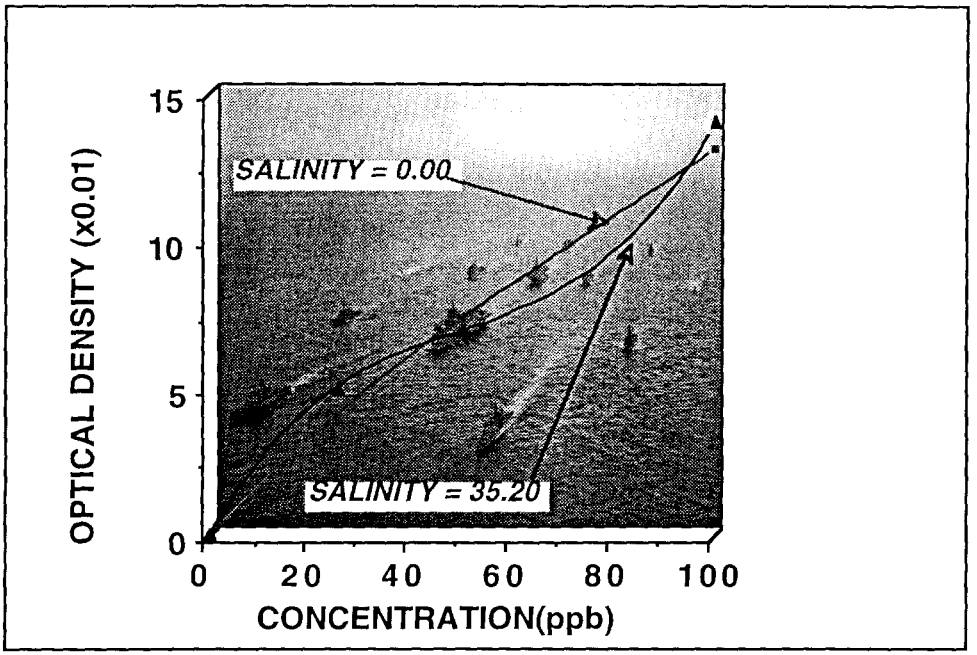


Figure 20. Comparison of the uptake of Cu(I) into the ionomeric membrane in the presence of seawater.

2. pH Affect

Figure 21 demonstrates the pH range over which the reaction of the BCS/BCP dye yields the best results.

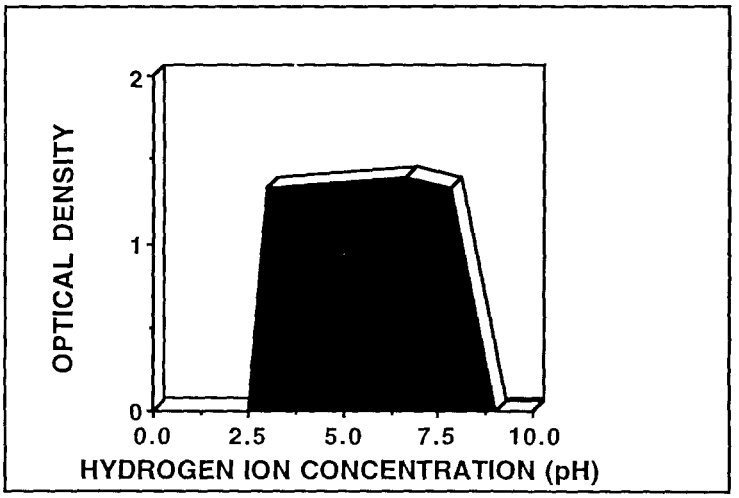


Figure 21. The pH range over which all copper is converted to copper (I) in this study.

3. Standardization Tests (Spectrophotometer)

Figure 22 is a series of curves scanned with the spectrophotometer. The values range from 10ppb to 2000ppb. The results of these tests indicated that the Standard Method (Clesceri et al 1998) was a reliable test to check the membrane analysis.

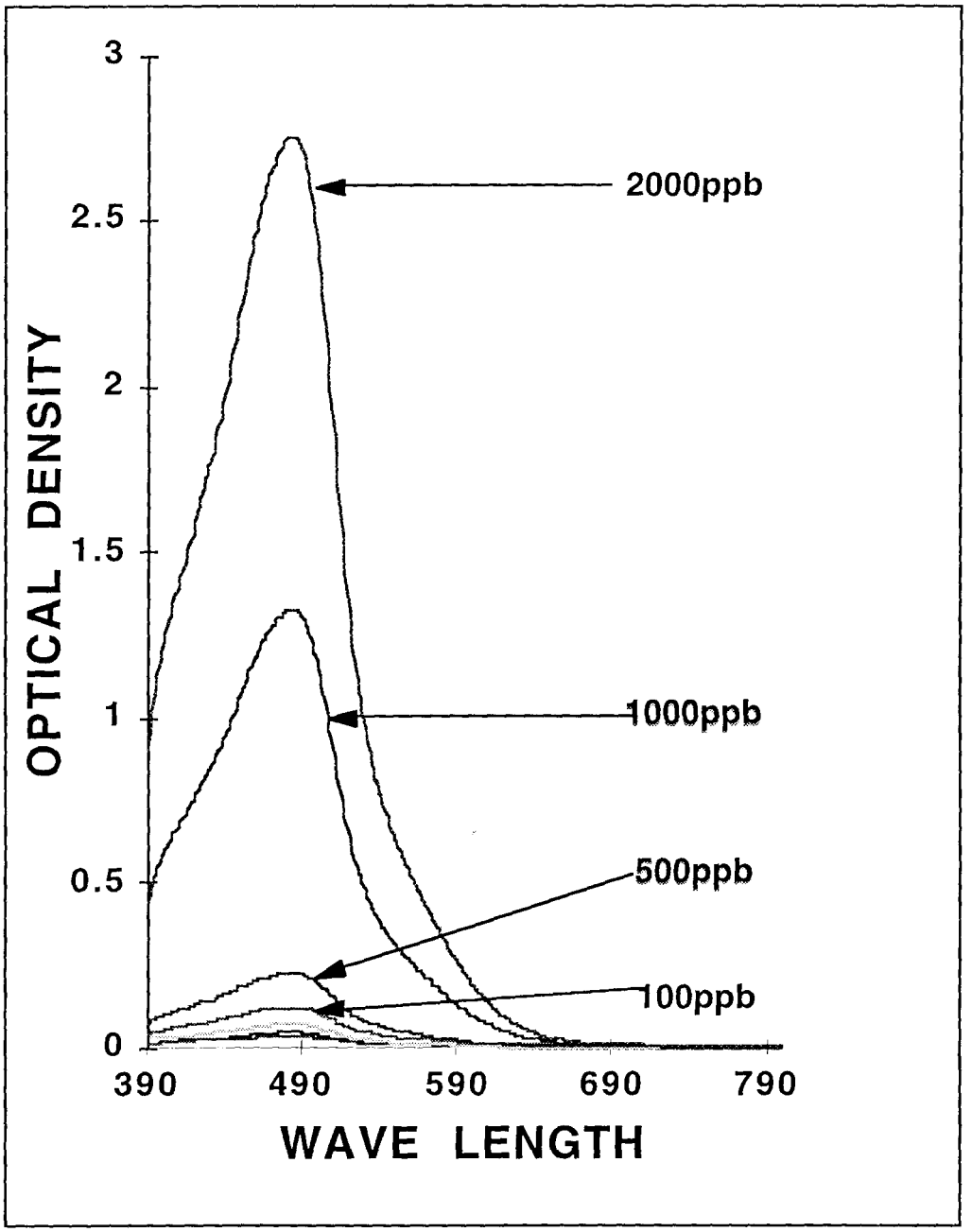


Figure 22. Scanning spectrophotometer plots of Copper (I) concentrations from 10 to 2000ppb.

4. Coupons - Source of Copper (I)

Ionic Copper [Cu(I) and Cu(II)] appears to quickly complex in the aquatic environment (Zirino and Yamamoto 1972, Steemann Nielsen and Wium-Andersen 1970, Alexander and Corcoran 1967). Providing a source of Cu(I) in a relatively natural state without changing the chemistry of the water is difficult. Copper containing anti-fouling coatings provide the vehicle for introducing Cu(I) into natural seawater. Glass coupons coated with copper containing anti-

fouling coatings are a source of Cu(I) and Cu(II) (Figure 12). In addition, after introducing copper (I), it is now possible to trace the conversion (oxidation) of the Cu(I) to Cu(II) (Figure 23 and 24). Addition of organic ligands (Figure 25), helps to trace the complexation the Cu(I) and Cu(II). This then yields an estimate of the short term toxicity of ionic copper (Steemann Nielsen and Wium-Andersen 1970, Edding and Tala 1996).

The coupons provided a source of copper (I) for

- leach rate studies in seawater, in distilled water, in pond water, and in chlorinated tapwater,
- copper (I) to copper (II) conversion,
- leach rate under water current movement, and
- probe studies.

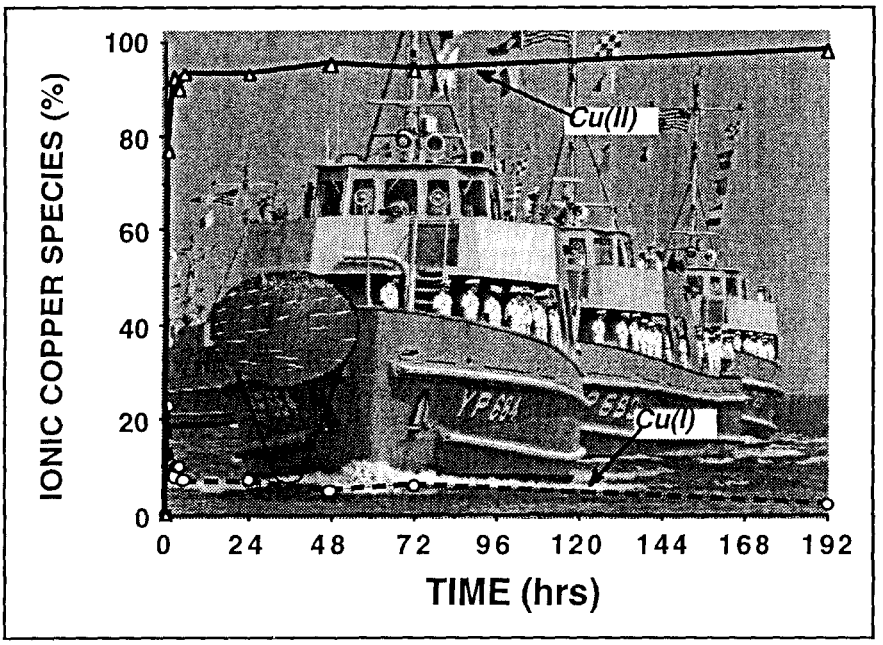


Figure 23. Leaching profile of Woolsey Neptune Red.

Though initially different in their leaching profile, both anti-fouling coatings tend to settle into a predictable leach rate.

Figure 25 demonstrates the effect of organic matter on ionic copper. Total available ionic copper appears to complex readily with organic matter (Leckie and Dåvis 1979), the processes of adsorption, absorption and complexion are present. Cu(I) and Cu(II) were introduced into samples containing 26-33 mg/L of suspended material (predominantly plankton). This is a normal ligand load for the area of the Chesapeake Bay used for the field test. The samples were left for a total period of 72 hours. After the first 16 hours, Cu(I) disappeared. Thirty two hours later (48 hours from start of test) Cu (II) was gone. Constant leaching by the antifoulant (Figures 23 and 24) will offset the loss of the ionic copper to ligands in the water column. Live plankton tends to protect itself from copper by complexing it with siderophores (Moffet et al 1997). This could be the reason for the higher complexation of the ionic copper by the live plankton.

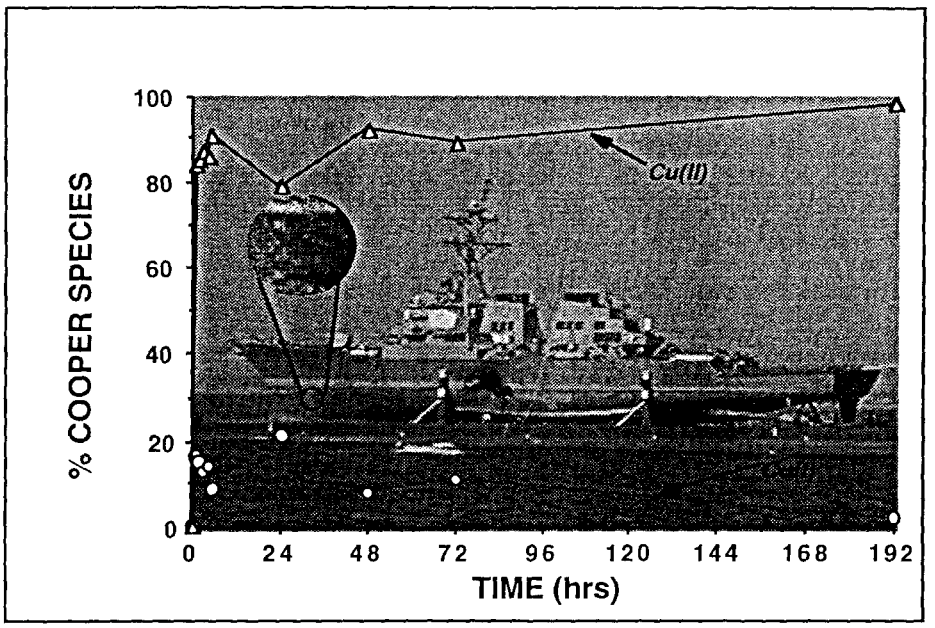


Figure 24. Leaching profile of Interviron.

Studies of copper (I) release in different types of water showed that the copper containing anti-foulant paint releases best in seawater. Leaching begins within the first 15 minutes of immersing the new coupon in seawater; 5 hours in pond water; and 16 hours in distilled water (Lamontagne et al 1998).

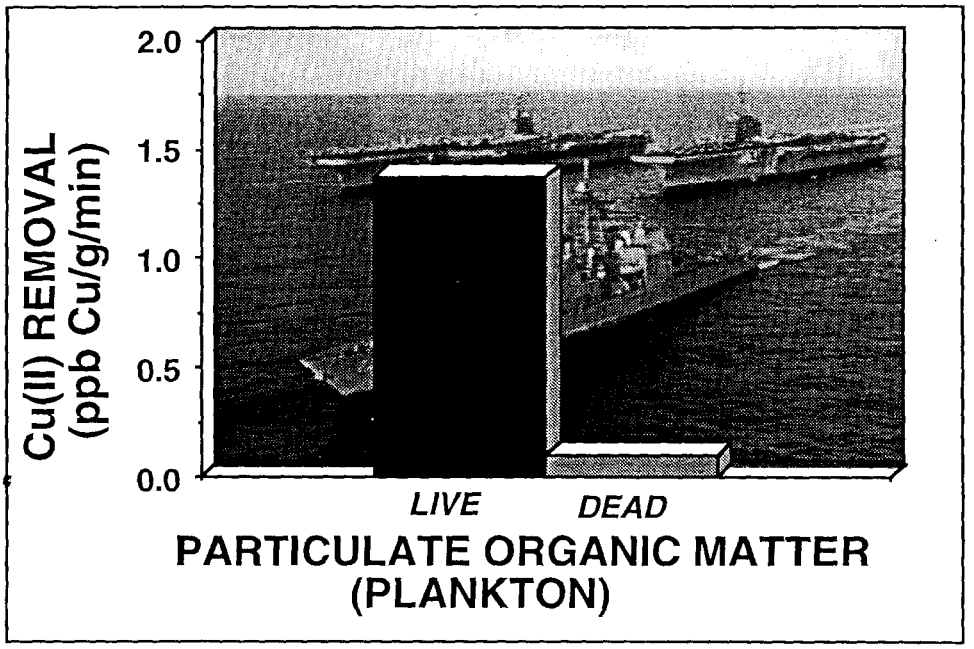


Figure 25. Organic ligands added to samples containing ionic copper.

5. Membrane Tests

5. Membrane Tests

Using the test scheme (Figure 16) BCP impregnated ionomeric Nafion 112 and 117 membranes were tested. The testings were mainly for

a) reaction conditions
- temperature
- pH
- salinity

b) response time
- NRL vs. LaMotte
- pre-treatment vs. post-treatment of membrane with Cu(I)

c) visual concentration levels [parts per billion (microgram liter^{-1})]

d) membrane processing
- quiescent vs. shaken
- razor cut vs. punched-out
- shape (round, rectangular, square)
- size
- preservation (ethyl alcohol, chloroform, KAF, de-ionized water)
- shelf life (membrane longevity, dye degradation)

5A. Reaction Conditions

For the most part, the temperature conditions of the experiments were kept in the range of 20°C to 25°C. A limited test of temperature effects on the dye complexation with Cu(I) was done. The experiments were conducted using de-ionized water and BCS at three widely separated temperatures (4°C, 24°C and 34°C). The results demonstrated that temperatures in the test range of 20°C to 25°C were best for development of the BCS/BCP dye (Figure 26).

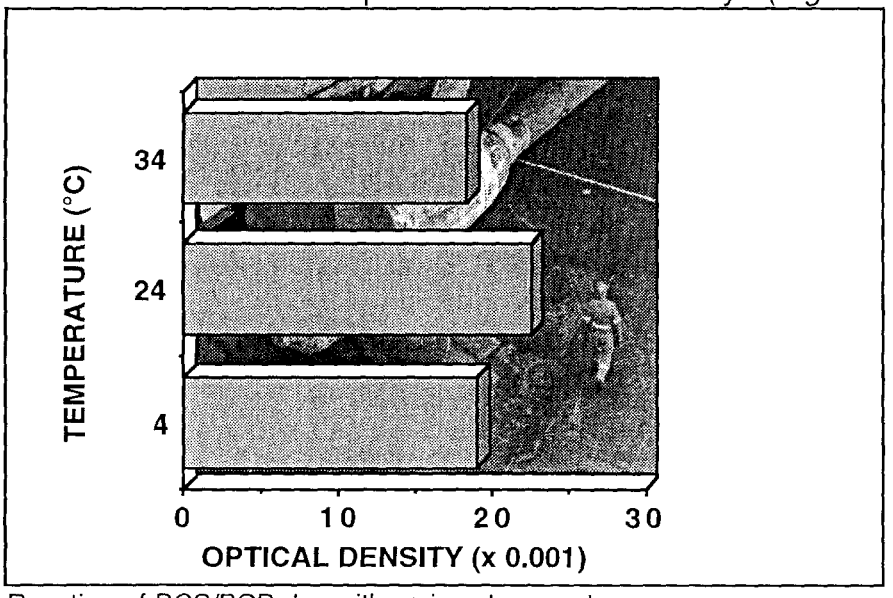


Figure 26. Reaction of BCS/BCP dye with various temperatures.

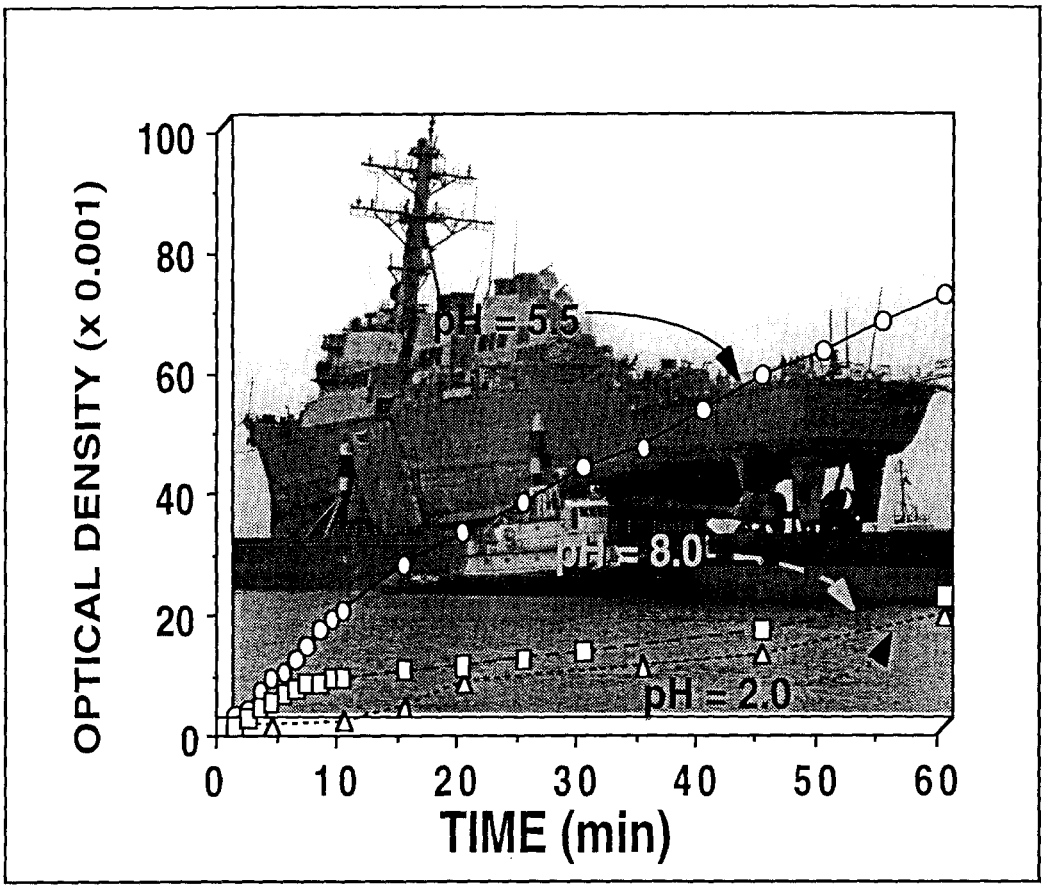


Figure 27. Uptake of Cu(I) by BCP impregnated ionomeric Nafion 117 membrane in various pH conditions.

Salinity (Figure 28) tends to affect the reaction of Cu(I) with the imbedded BCP in the ionomeric membrane in the low range (<100ppb). There appears to be a slowing of the reaction initially. However, over all the curves are similar between de-ionized water and seawater and the curves conform to Beer's Law in the range of 10 to 1000ppb.

In a further analysis, the uptake of Cu(I) by the BCP imbedded ionomeric Nafion 117 membrane was compared to tap water containing chlorine generated from HTH (calcium hypochlorite) in the free chlorine range of 1-4ppm. Over a time period of 24 hours, the chlorine containing water slows the BCP reaction with the Cu(I) (Figure 28). The hypochlorite would be a competitive oxidant with the Cu(I).

Figure 28 has a view of the overall 24 hour reaction profile of the Cu(I) in deionized water, seawater and tap water containing HTH. The magnified portion of Figure 28 is demonstration of the beginning of the reaction . Tap water containing HTH appears to retard the initial reaction of the Cu(I) with the BCP imbedded Nafion 117 membrane.

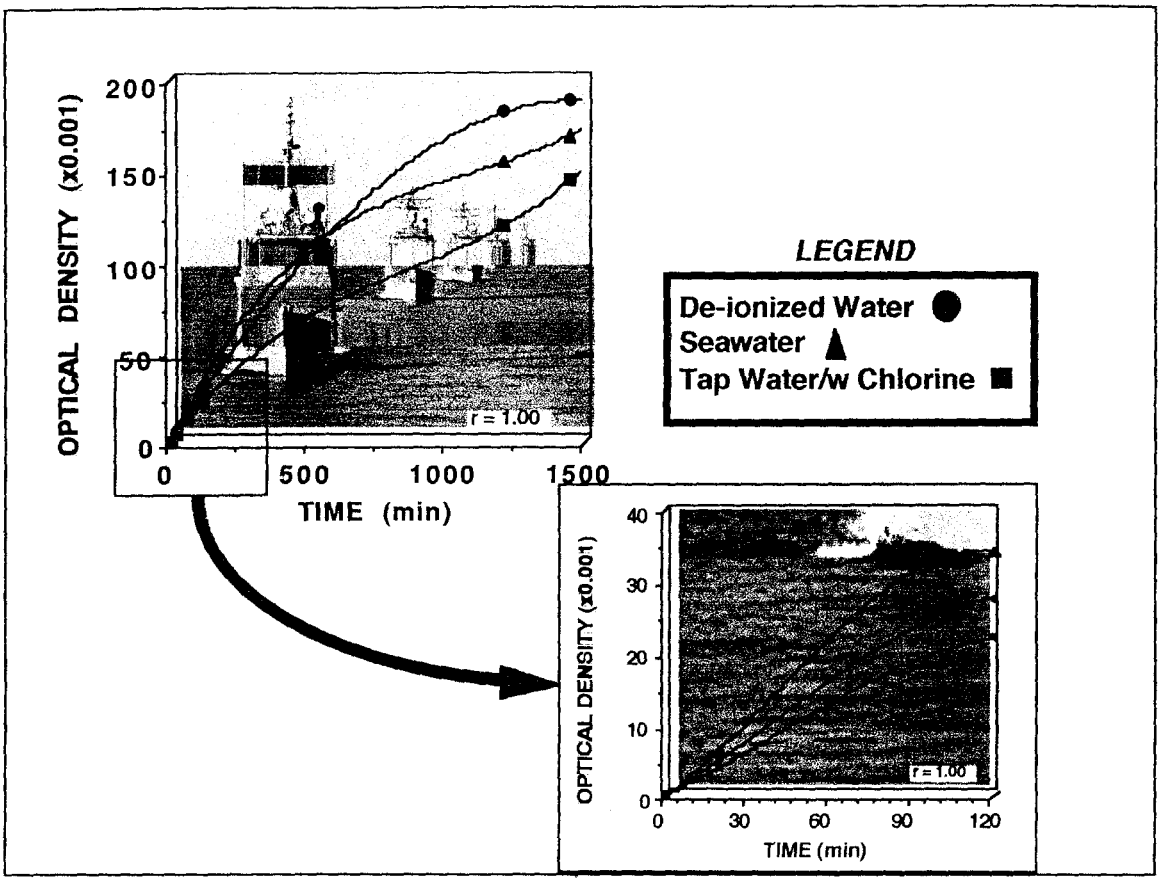


Figure 28. Reaction of Cu(I) with BCP imbedded in ionomeric Nafion 117 exposed to different types of chemical conditions in the water.

5B. Membrane Response Time

Birch et al (1995) reported on the use of polymers in the environment. The techniques were better than electrochemical methods. They stated that "...optical techniques ...(are) more suitable because of the convenience of using optical fiber coupling." This study tests the hypothesis that BCP attached to the Nafion 117 ionomeric polymer is a reliable optical sensor system for measuring copper in seawater.

One of the key features needed for this method is an ability of the BCP to react quickly with the Cu(I) imbedded in the ionomeric Nafion 117 membrane. In initial studies, optical measurements of the imbedded membranes using a colorimeter and an optical fiber spectrophotometer showed reaction of the BCP dye to occur within 1-15 minutes depending on the size of the membrane. The smaller the imbedded membrane, the quicker the color developed. For purposes of developing a comparator that could be visually read, the reaction time takes longer. At present the full development time appears to be 24 hours. The development of the color appears to be rate limited by diffusion into the membrane. A similar experience was detected in using BCP impregnated liquid Nafion 117 coating a C18 bead placed on the tip of an optical fiber spectrophotometer (Lamontagne et al 2000). Figure 29 is the mean of the data

developed from repetitive testing of the reaction times using 1000ppb of Cu(I) in de-ionized water at a temperature of 25°C. Initially, there is a reaction, which then slows for a period before climbing to a plateau.

Figure 30 demonstrates the various concentrations of Cu(I) with the response time. Each concentration curve follows a similar pattern with a plateau underway by 24 hours.

Figure 31 compares the response of the membrane probe imbedded BCP with 1000ppb Cu(I) over a 24 hour period. The seawater has a salinity of 35.2ppt and the experiment was run at 25°C. There is a similar response.

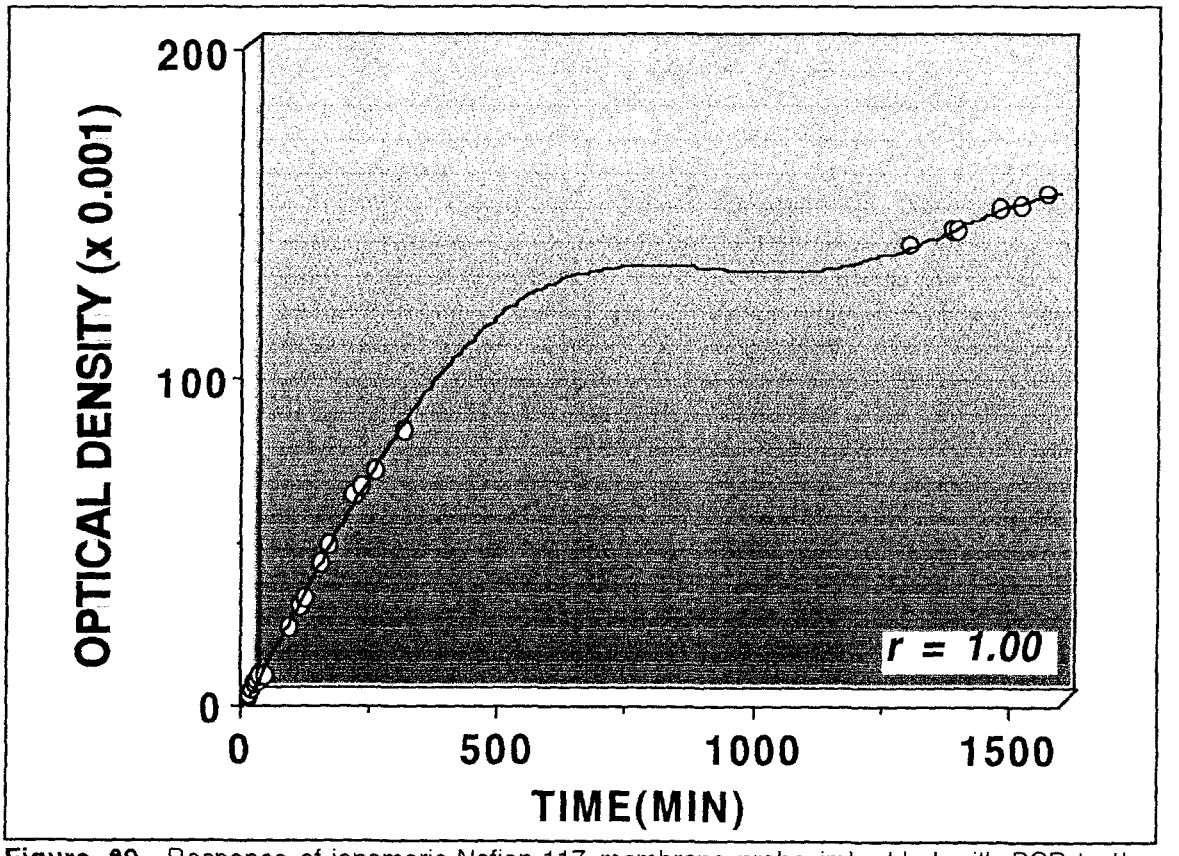


Figure 29. Response of ionomeric Nafion 117 membrane probe imbedded with BCP to the presence of 1000ppb of Cu(I).

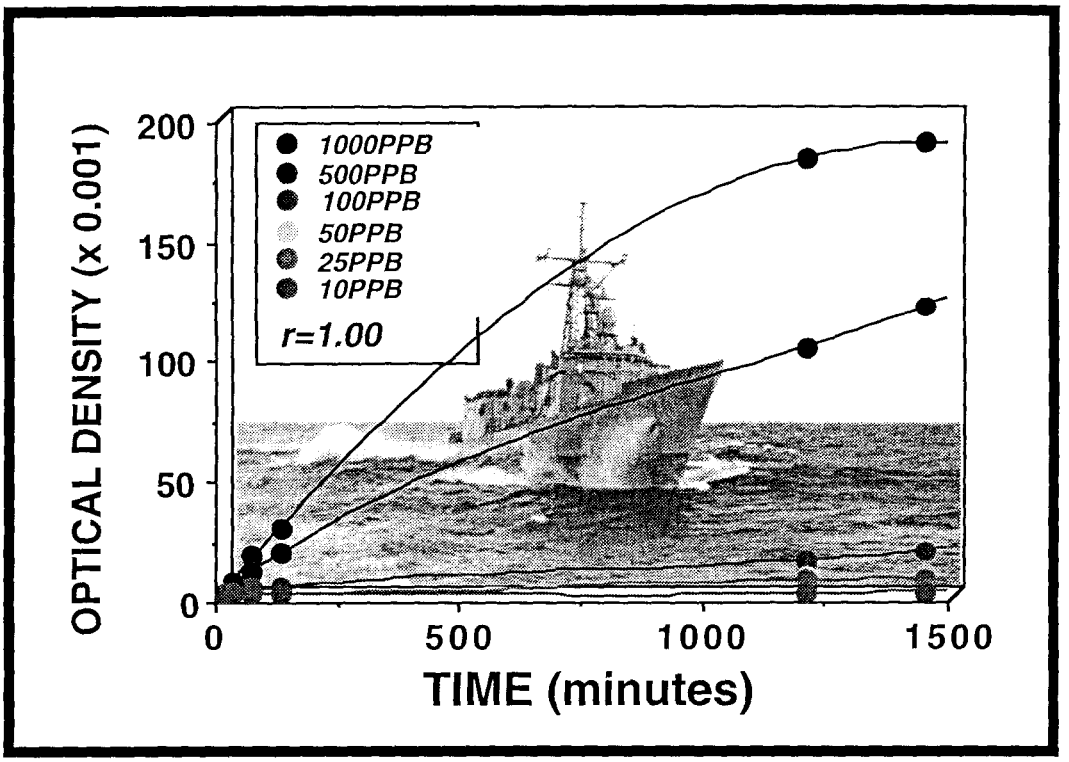


Figure 30. Response of the membrane probe with different concentrations of Cu(I).

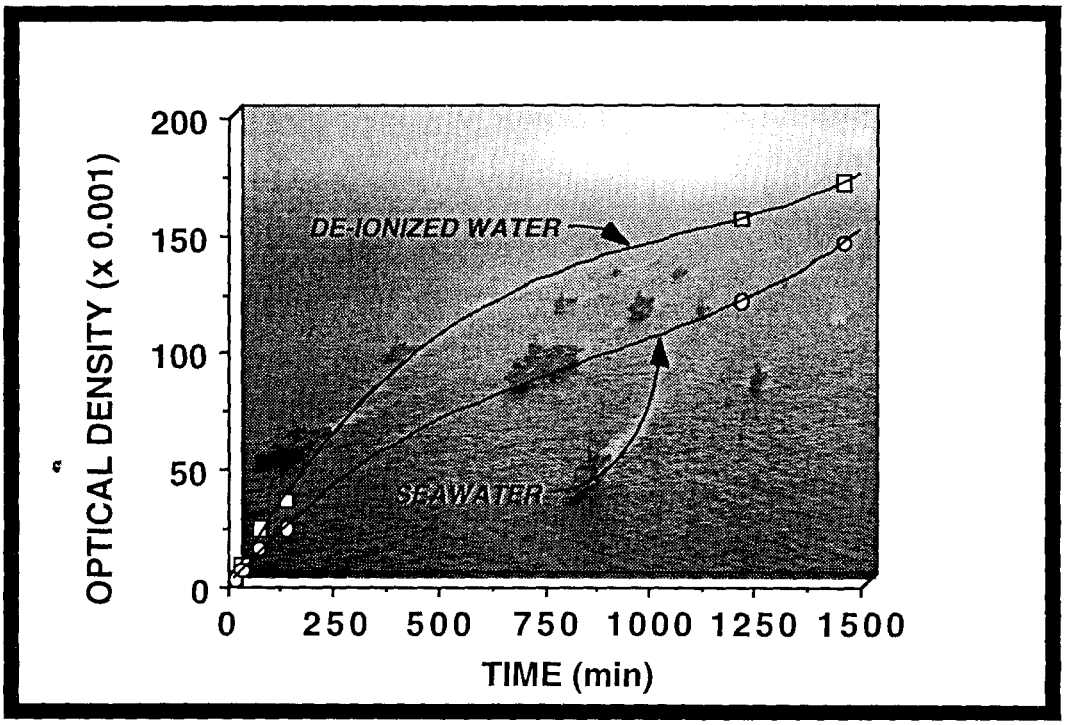


Figure 31. Response of the membrane probe with de-ionized water and natural seawater to 1000ppb of Cu(I).

5C. Visual Concentration Levels

The membrane probe with the imbedded BCP reacts with the Cu(I). The Cu(I) changes the optical properties of the BCP and creates an orange color. The intensity of the orange color is visible and in proportion to the concentration of the Cu(I). Figure 32 represents the membrane probes with concentrations of Cu(I) between 10ppb and 1000ppb. Below 10ppb and above 1000ppb, the membranes are hard to read without optical instrumentation.

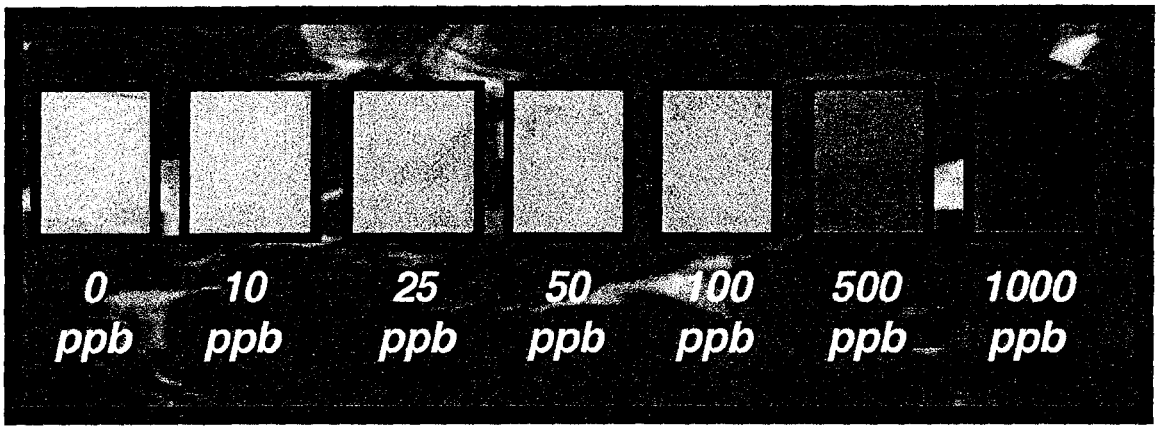


Figure 32. Comparison of orange intensity from actual membranes placed in varying concentrations of Cu(I) for 24 hours at 25°C in de-ionized water on an orbital shaker revolving at 100 revolutions/ minute.

In an effort to determine how the membrane probe works, an atomic force microscope was used. Figure 33 represents the surface configuration of the treated and activated ionomeric Nafion 117 membrane probe (A), the untreated membrane probe (B), and an atomic force microscope. Figure 33A has the scale used for studying the membranes.

The ionomeric Nafion 117 membrane probe develops a structure (Figure 34) which changes the surface configuration of the membrane. Each of the images are at the same scale and are extracted portions of the original scan. This analysis was performed using an atomic force microscope with a floating head stylus.

Another experiment used a Minolta Chroma Meter CR-200 to determine the increase in color density as the membrane uptakes Cu(I). At low concentrations the meter sensed a change (Figure 35). The Chroma-Meter allows for a comparison of color density to the visual perception of the human eye. For a "probe" to be useful, the lower concentrations need to be quantified and related to human color perception. Comparing the results of Figure 35 to Figure 32 would indicate that actual visual perception of the concentrations in the range of 10-50ppb would depend on an individuals visual acuity. Further work in this area is needed to enhance the development of the color. The

colorimeter and the Chroma Meter readily see the change but using visual assessment is more difficult.

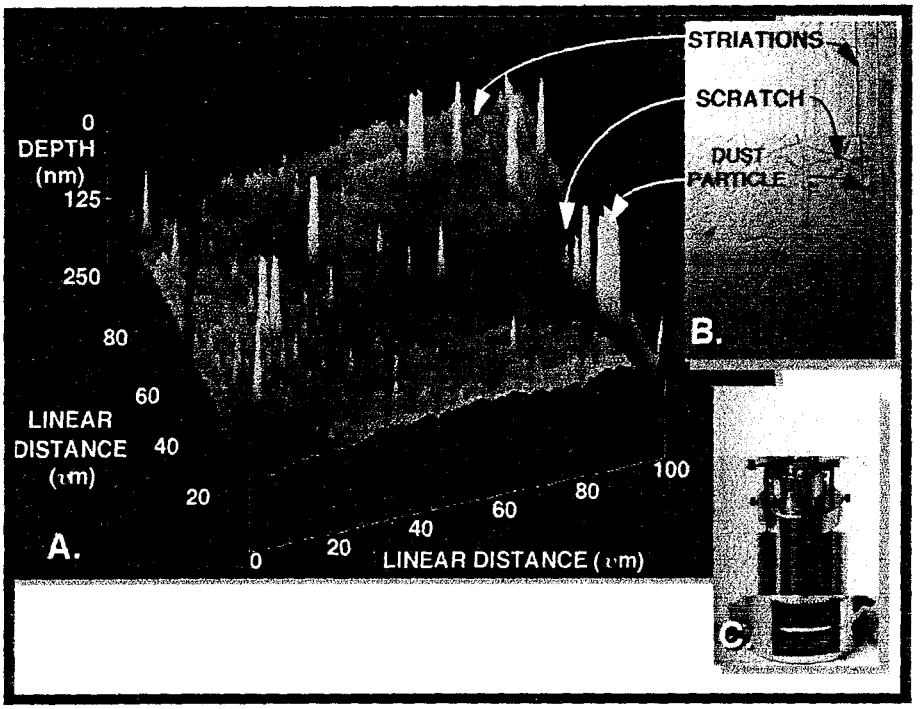


Figure 3 3. The results of using an Atomic Force and a Light microscopy to determine how the ionomeric Nafion 117 membrane probe responds to treatment. A=Atomic Force analysis of membrane surface with BCP imbedded and treated with Cu(I); B=Light microscope view of membrane surface before cleaning and chemical treatment; C=Atomic Force Microscope (AFM).

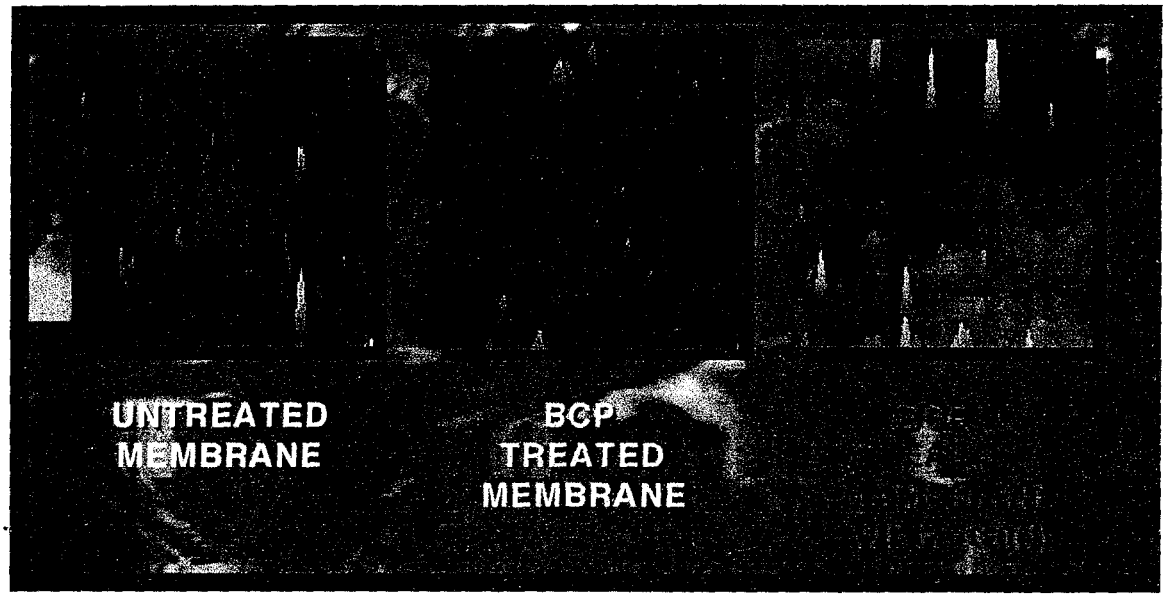


Figure 3 4. The change in the ionomeric Nafion 117 membrane as it is imbedded with BCP and then treated with Cu(I). All images are from an Atomic Force Microscope (AFM) with a floating head stylus and use the same scale and at the same scale(see Figure 33).

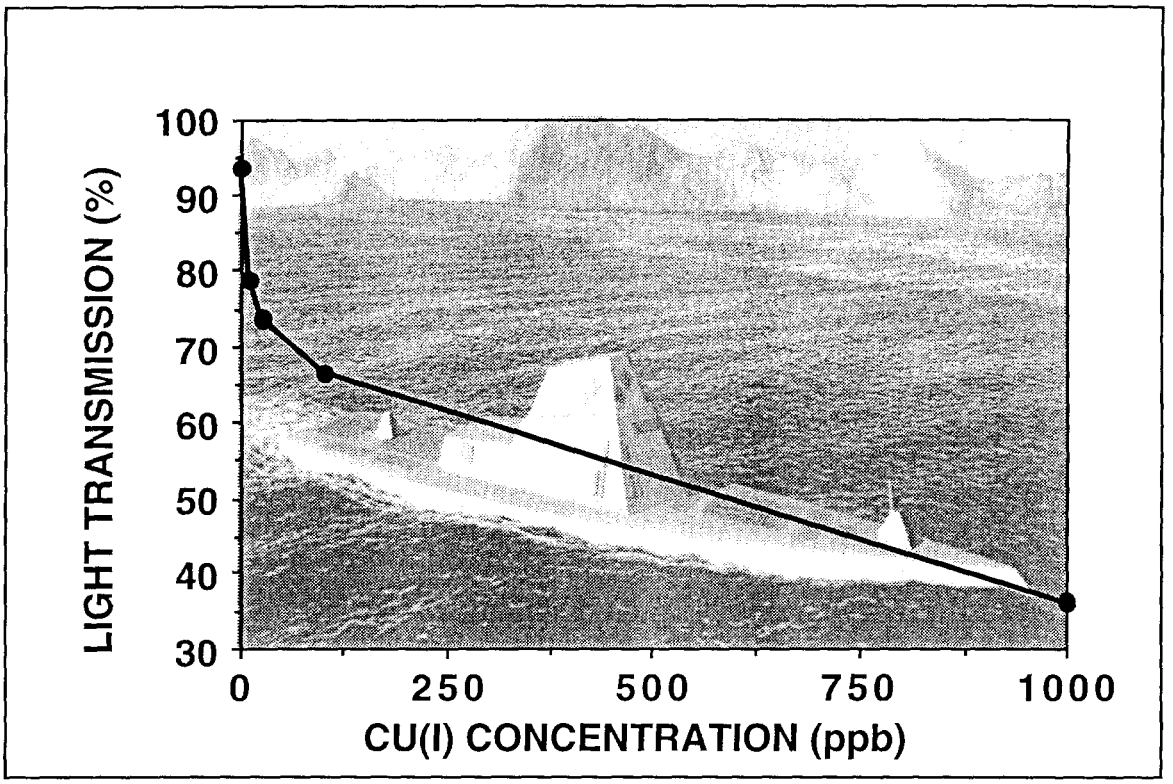


Figure 35. Color density measurements of membrane developed with Cu(I) using a Minolta Chroma Meter CR-200.

5D. Membrane Processing

The LaMotte Chemical Company was tasked to provide membranes for testing according to the method discussed in Section II. Figure 36 represents a comparison of the response by the membranes from both laboratories. In essence both membranes appeared to respond in a similar manner. At varying Cu(I) concentrations (10, 25, 50, 100, 500, 1000ppb), the membrane response curves and response times were similar.

An additional series of experiments were performed to test whether it was effective to shake the samples containing Cu(I) and a membrane, or leave the membrane to stand. Figure 37 was developed to demonstrate that moving the test solution containing the Cu(I) past the membrane enhanced uptake, especially at lower concentrations. Hypothetically, a diffusion depletion zone may form near the membrane surface which will slow movement of Cu(I) into the membrane probe. These data suggested that in any membrane dip probe system, agitation of some sort is necessary to achieve the maximum uptake of ionic copper.

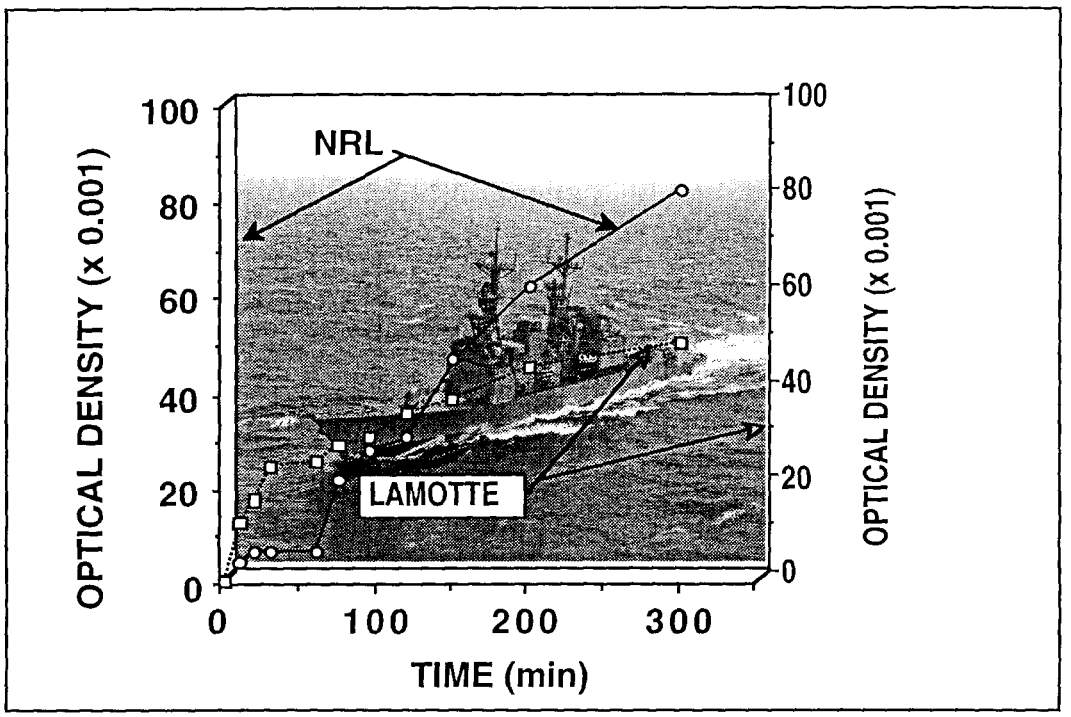


Figure 36. Comparison of the response of membranes made by 2 different laboratories. Cu(I) concentration is 1000ppb and the test was made in deionized water at a temperature of 25°C.

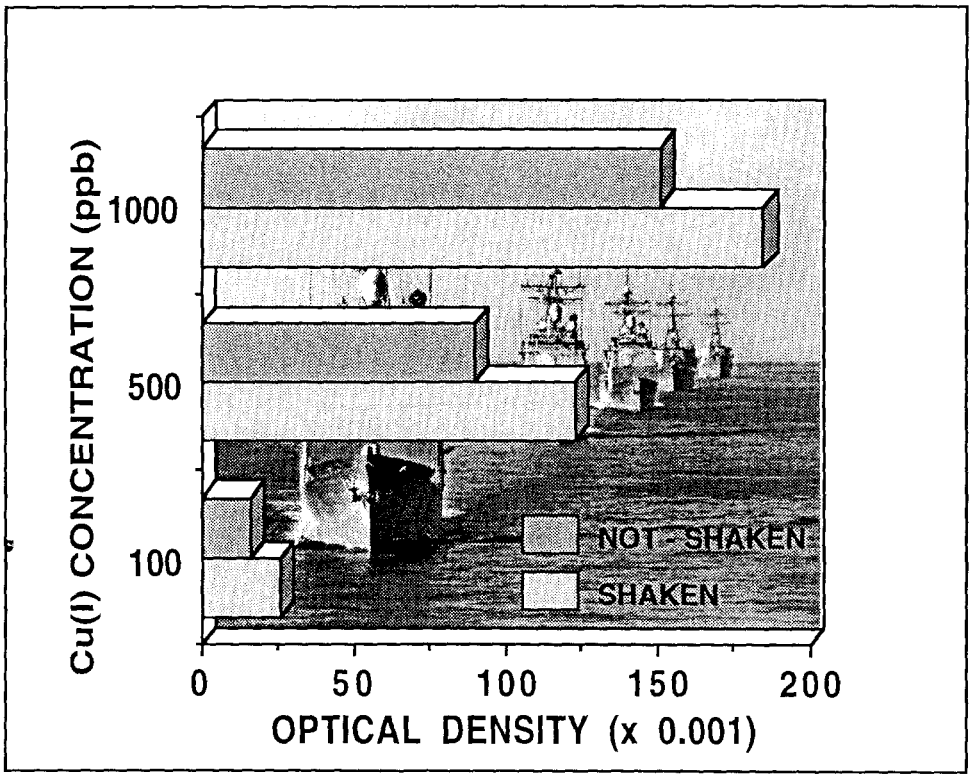


Figure 37. Comparison of membranes shaken during uptake of Cu(I) with those not shaken. Test duration=24hrs.; Shaking Oscilation=100rpm at a temperature of 25°C.

Another effect on the uptake of Cu(I) into the membrane probe was the method used to cut the probe into a shape and size for reading. Figure 38

demonstrates the difference between razor cutting the membrane before and after imbedding the BCP and a machine punched membrane made before activating with BCP. Cutting the membrane before activation appears to lessen the membrane probe's capability to detect Cu(I). In theory, what may be happening is the process of cutting is sealing the channels in the ionomer where the BCP will locate. For whatever reason, the membrane probe needs to be cut after imbedding the BCP.

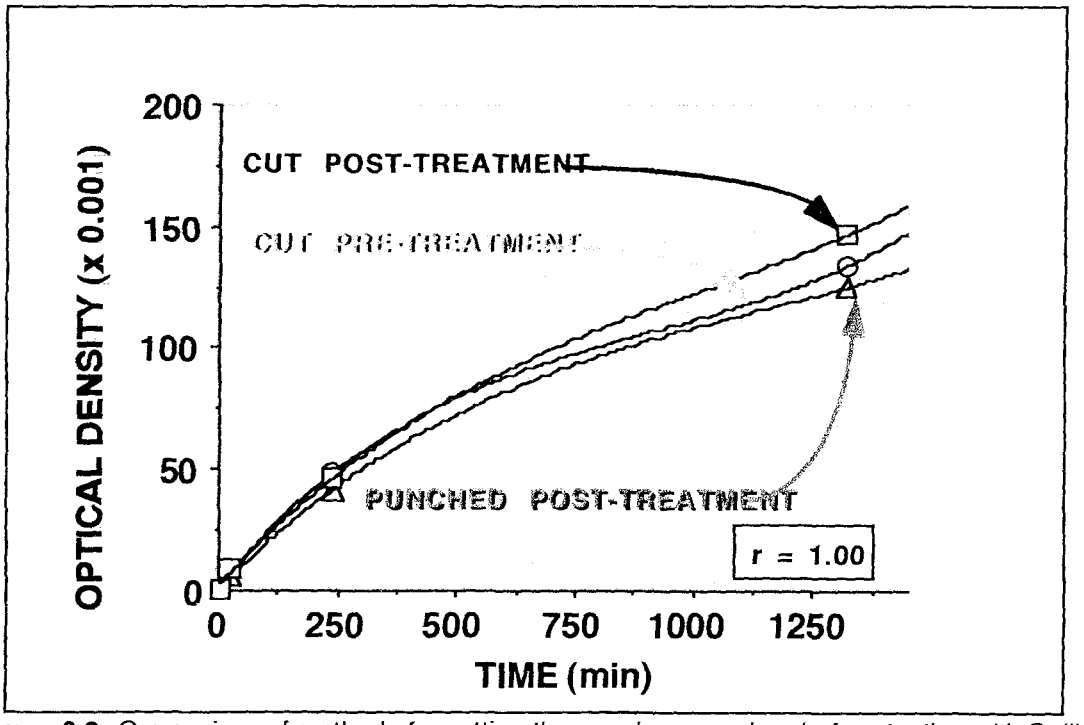


Figure 38. Comparison of methods for cutting the membrane probes before testing with Cu(I).

Figure 39 represents a series of tests on the membrane area size. The larger the area, the less intense was the color development.

Once the membrane is developed, it appears it can sit on the shelf for at least a year without degradation in capability. In addition, once the membrane probe is activated with Cu(I), you will get a degradation in color of about 0.2% per day. This is based on activating membranes with Cu(I), reading them and then placing them in distilled water for a year. This is within the parameters described by Blair and Diehl(1961) for the degradation of BCP over time.

Since the ionomeric membrane Nafion 117 is organic and is imbedded with the organic dye BCP, there is a possibility of infection by fungi and bacteria. This did not seem to be a problem with the membranes kept in de-ionized water. However, three preservatives (ethyl alcohol, chloroform and Kathon CG [5-chloro-2methyl-4-isothiazolin-3-one and 2-mthyl-4-isothiazolin-3-one]) were tested to see if they affected the performance of the imbedded membrane

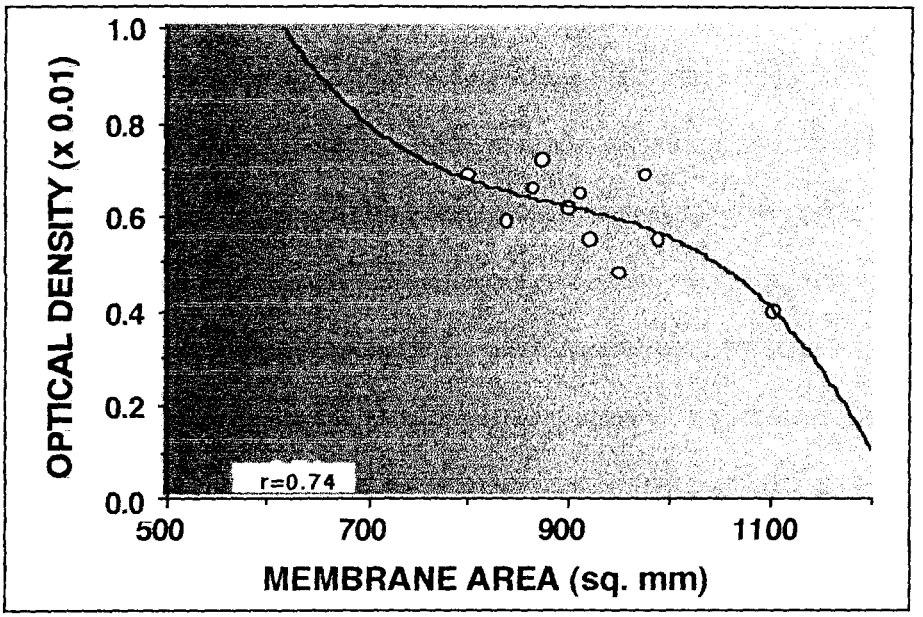


Figure 39. Comparison of membrane test areas. The larger the area the less intense the color development.

probe. Figure 40 is the result of testing the membranes stored in 3 preservatives, seawater at 35 parts per thousand (ppt), and de-ionized water. Ethyl alcohol and Kathon CG appear to affect affect the uptake of the Cu(I). This is seen when comparing the shape of their curves to that of seawater and de-ionized water. Ethyl alcohol appears to depress the uptake of Cu(I) into the membrane.

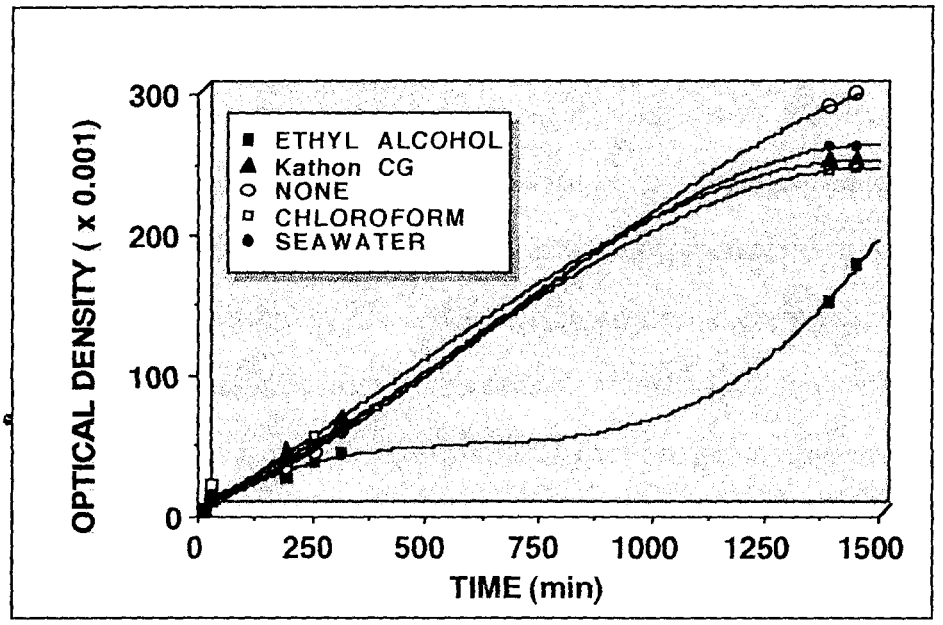
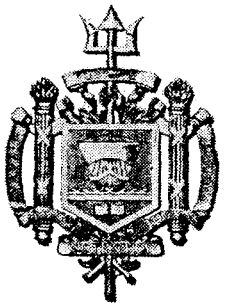


Figure 40. The effect of preservative on the membrane and its ability to uptake Cu(I).

Chloroform performs similarly to seawater and de-ionized water. Based on this data, if a preservative is needed, chloroform appears to have the least affect on the uptake of Cu(I) into the membrane.

Additional and similar tests were performed on the thinner ionomeric polymer membrane, Nafion 112. It was found that the Nafion 112 is subject to wrinkling and thus uneven distribution of the imbedding BCP. This thinner membrane is difficult to handle at this time and thus was abandoned in favor of the more robust Nafion 117.

CONCLUSIONS









IV. CONCLUSIONS

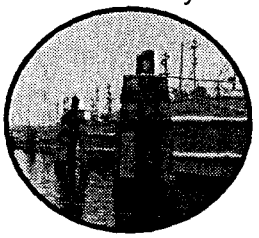
Copper (I) is a potentially toxic trace metal controlled in the environment by the chemistry of the water column (pH, temperature, salinity, ligands). An anti-fouling coating containing Cu(I) presents a challenge to the environment because it is deigned to leach a toxic amount continuously over a period of time. This then is the pesticide that kills or prevents the attachment of organisms to a ship hull. It is also the source of introduced Cu (I) to the environment far above the "natural" levels reported by Moffett et al 1985. The ability to monitor the concentration of Cu(I) around Naval vessels as well as monitoring the input and output of this species in marine environments that are under the control of the DoD is very important.

This study has shown that: 1) the use of the chemical procedure using BCS (the Standard Method) affords both a total copper and copper (I) analysis; 2) BCP can be imbedded in Nafion 117 by both small batch laboratory procedures and small scale-up using commercial equipment with very similar results; 3) the chemical procedures for analysis are robust and reproducible; 4) there is a knowledge base for continuing and expanding the copper (I) studies particularly in the area of response time and color intensity.

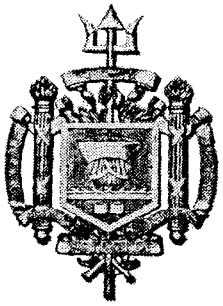
The robustness and sensitivity of the membranes has been shown for a wide range of temperatures, salinities, pHs, and ligands. This robustness and sensitivity has also been shown for membranes that have been stored in various solutions including de-ionized water, seawater, and various preservative solutions. The ability to maintain membranes in an operational state for long periods of time bodes well for the possible pre-packaging of membrane dip probes for off-the-shelf use.

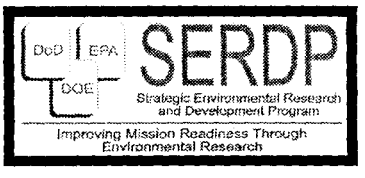
For implementation of this research into an easily used membrane dip probe that is inexpensive and disposable, a commercial production run of membranes had to demonstrated. The membranes produced by LaMotte Chemicals were such a demonstration. The scale-up was small (1000 membranes), but the membranes were produced in a commercial facility using production equipment. The results from these membranes were very similar to those obtained from small batch laboratory procedures.

Further testing needs to be performed in ways to enhance the color of the membrane for use in a membrane dip probe testing system. Visual color recognition and comparison in samples below 50 ppb is very dependent upon the visual acuity of the individual user. An alternative to color enhancement studies would be to examine the use of hand held fiber optic devices as a means of overcoming individual visual acuity issues.



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V. LITERATURE CITED

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VI. ACKNOWLEDGMENTS

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